PLANT PHYSIOLOGY

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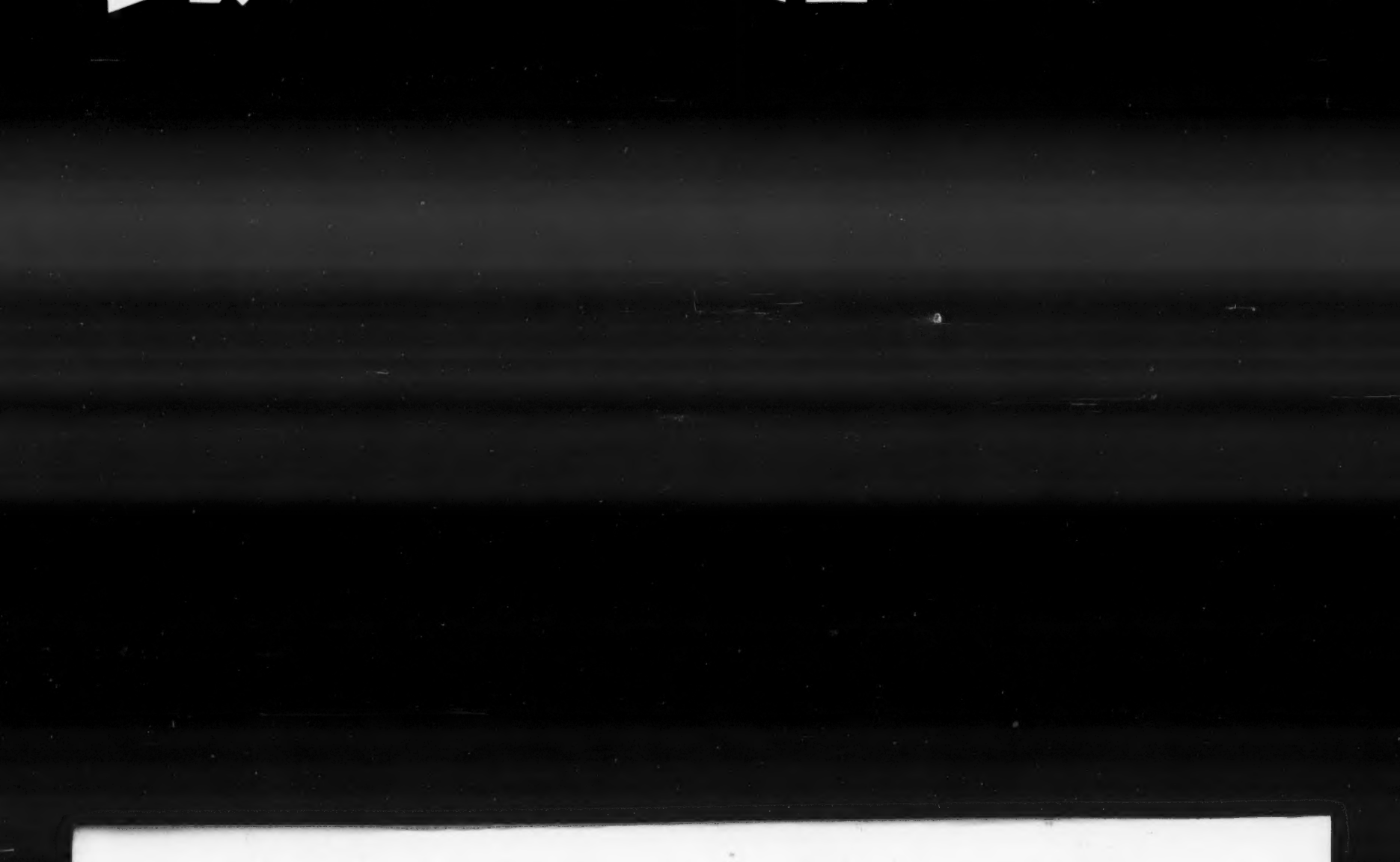
ERRATA

Page 16, line 4 from bottom, for "thorough" read through.
Page 36, line 21, for presures read pressures.
Page 36, line 23, for presure read pressure.
Page 41, line 11, for coresponds read corresponds.
Page 68, reference 38, for exacten read exakten.
Page 69, line 11, for mongraph read monograph.
Page 124, experiment no. 16, for last figure + 11 read + 101.
Page 124, experiment no. 16, for average + 41 read + 53.
Page 124, experiment no. 17, unexposed side, eighth column, for 43 read 48.
Page 153, line 8, for indicates read indicate.
Page 192, line 13, for are read is.
Page 208, line 4, for easly read easily.
Page 208, line 11 from bottom, for activited read activated.
Page 222, line 9, for was read were.
Page 222, line 10 from bottom, for have read has.
Page 313, line 1 of text, for infection read inflection.
Page 413, line 12, for contains read contain.



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PLANT PHYSIOLOGY

JANUARY, 1930

THE RELATION OF TEMPERATURE AND THE PARTIAL PRESSURE OF OXYGEN TO RESPIRATION AND GROWTH IN GERMINATING WHEAT*

WARREN B. MACK

(WITH SEVEN FIGURES)

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Introduction

GENERAL STATEMENT.—The present introductory discussion and the experimental study reported in this paper deal with the influence of temperature and of the concentration or partial pressure of oxygen in the surroundings, on the rate of evolution of CO_2 by higher plants in darkness and with adequate water supply. There is a considerable literature on the relation of higher plants to oxygen supply, and the literature on the influence of temperature and oxygen supply upon respiration in lower plant forms (such as bacteria, yeasts and fungi) likewise is extensive, but with the latter aspect of the subject we need not be concerned here.

Respiration rates may be ascertained in terms of the absorption of oxygen and of the disappearance of the respired plastic materials, such as carbohydrates, etc., but the rate of CO_2 production furnishes the most convenient and practical index of respiratory activity, and it is the index that has generally been employed in experimentation in this field; therefore measurements on CO_2 production will here be generally considered as indications of the respiration rate. The respiration rate is so dependent on temperature that no very extensive study of the process could be made without the inclusion of temperature among the measured variables and it is necessarily included here. The influence of light on respiratory activity appears to be slight in all cases where such influence seems to occur, excepting those instances where light operates through the photosynthesis of carbohydrate, in which instances it cannot be studied directly; indeed the experimental problem of the physiological control of respiration in green plants in light is a very special and difficult one. The water content of the respiring tissues is known to influence respiration very markedly, but only in its lower ranges; it may consequently be left out of account when the respiring material is well supplied with water. In a similar manner the influence of the partial pressure of CO_2 in the surroundings may be neglected when the gaseous products of respiration are allowed to escape freely so as not to accumulate within the cells to any significant extent. There are of course innumerable unessential substances that influence respiration, growth, etc., if present in suitable concentrations in the surroundings, their effects being sometimes to accelerate respiration and sometimes to retard it, according to their natures and their concentrations. Any of these substances may be made the subject of special study after the fundamental temperature-oxygen relations of respiration have been worked out for a given plant material. When such substances are absent or in sufficiently low concentrations it is not necessary to consider them.

After the study here reported had been completed another series of experiments was planned and carried out under similar conditions but in-

cluding a constant partial pressure of ethylene, an unessential substance that has recently assumed considerable importance in respect to the artificial treatment of fruits, vegetables, etc., for the market. The results of the ethylene experiments are reserved for another paper, although some aspects of the problem of ethylene influence are briefly considered in the present contribution.

The process of respiration is fundamental in all vital activity and increased knowledge concerning it is needed in many parts of the field of plant physiology and ecology, including agronomy, horticulture, forestry, etc. The influence of environmental conditions on respiration is inevitably involved whenever the more general processes of growth and development are being studied, as in discussions of seed germination [HUTCHINS, (**18**)], the behavior of roots in soils [CANNON, (**7**)], and the behavior of fruits, tubers, roots, cuttings, etc., in storage.

Because the respiration process as a whole is closely related to vitality in general and to the various kinds of growth and developmental changes that occur in a plant (such as enlargement, ripening, etc.), it is always desirable to give some attention to growth and development when respiration is to be studied. This is especially true when germination processes and ripening processes are considered, in which development and respiration are so interrelated as to be incapable of separation, even in theory and definition. Consequently the present paper includes some discussion of the relation of growth to oxygen supply and temperature.

A brief résumé of our knowledge concerning the influence of oxygen concentration and temperature upon the rate of evolution of CO_2 by higher plants in darkness and without water deficiency is presented below.

RELATION OF OXYGEN PRESSURE TO CO_2 PRODUCTION.—KOSTYTSCHEW, in his recent special monograph on plant respiration (**25**, p. 18–19) confines his review of the literature bearing directly on the influence of oxygen concentration to a single paragraph. His general conclusion is that, except for plants under extreme conditions, as when they are in nearly pure oxygen or in an atmosphere with less than 1 or 2 per cent. of oxygen, the concentration of that element has little effect on the rate of respiration. PALLADIN (**32**, p. 215) makes the brief statement that the partial pressure of oxygen in the surrounding atmosphere influences plant respiration without changing the value of the respiratory ratio. This statement is probably based on the results of GODLEWSKI'S work (**12**). KOSTYTSCHEW cites the work of DE SAUSSURE, WILSON, JOHANNSEN, and STICH, which seems to show that changes in external oxygen pressure, even to a considerable degree, effect little change in the rate of CO_2 production. But the results secured by GODLEWSKI point to a different conclusion.

In GODLEWSKI's experiments (**12**) on radish seedlings a gradually diminishing concentration of oxygen was brought in an enclosed space, by the respiration of the seedlings contained therein, and the rate of CO_2 production steadily decreased as the partial pressure of oxygen was reduced. It might be thought, however, that this reduction in respiratory rate may perhaps have been related to accumulation of products of physiological activity as well as to the lowering of the oxygen pressure.

In WILSON's experiments (**46**) sunflower seedlings gave off as much CO_2 when in a mixture of 1 volume of ordinary air and 4 volumes of hydrogen as they did when in ordinary air. When, however, the seedlings were changed from air to a mixture of 1 volume of air and 19 of hydrogen, the rate of CO_2 production fell from 18.6 mg. per hour to 12.1 mg. per hour, and when these same seedlings were returned to air the rate rose to 17.8 mg. per hour. The time of exposure was from 1 to 1.5 hours. In STICH's experiments (**43**) on wheat seedlings and with an exposure time of one hour, a change in the concentration of oxygen in the surroundings from 20.8 per cent. (ordinary air) to 2.0 per cent. caused no change in the rate of CO_2 production. JOHANNSEN (**19**) found that an increase in the total pressure of ordinary air up to 2 and even to 5 atmospheres produced no effect on the carbon dioxide output of pea seedlings. Respiration of pea seedlings in nearly pure oxygen at ordinary pressures was no more rapid than in air; maize seedlings, however, produced CO_2 a little more rapidly when surrounded by nearly pure oxygen. When sunflower seedlings that had been in air were tested for 45 minutes in a mixture of 1 volume of air and 20 of hydrogen there was a decrease in the rate of evolution of CO_2 and when they were returned to air at the end of the 45-minute period the rate increased slightly, but the original rate in air was not regained in three-quarters of an hour after the return. The relative rates for air, air-hydrogen mixture, and air in succession were 14, 9, and 10, respectively.

It is interesting to note that WURMSER and JACQUOT (**47**) found a very striking dependence of the respiration rate of a kelp (*Laminaria saccharina*) on the oxygen content of the surrounding sea water. For oxygen contents of 5.2, 6.0, 12.0, 20.0, and 26.0 cc. per liter of water, the relative rates of CO_2 production were 1.0, 1.6, 2.4, 3.1, and 6.3, respectively, being approximately proportional to the oxygen content of the water. HEE and BONNET (**15**), however, in similar studies on higher plants, found only slight increases in the respiration of water-weed (Elodea sp.) and water milfoil (*Myriophyllum spicatum*) for increases in the oxygen content of the surrounding water from approximately 3 to 20 cc. of oxygen per liter. The time of observation for each oxygen concentration was from 3 to 4 hours, and the same individual plant was used throughout the series. In the field of

animal physiology, AMBERSON, MAYERSON and SCOTT (1) found that, for the lobster (*Homarus americanus*), the annelid worm (*Nereis virens*), the king crab, (*Limulus polyphemus*) and the blue crab (*Callinectes sapidus*), the rate of oxygen consumption was directly proportional to the oxygen tension of the sea water surrounding them, for a wide range of oxygen tensions. For a shrimp (*Palaemonetes vulgaris*) and a squid (*Loligo pealei*) the rates of oxygen consumption were directly proportional to oxygen tension of the sea water when this was below 50 per cent., and 30 per cent. of oxygen saturation, respectively. Above these values, however, the oxygen consumption of these two forms was found to be independent of the concentration of oxygen. NOMURA (31) found that oxygen consumption by a holothurian sea-slug (*Caudina chilensis*) was directly proportional to the oxygen tension of the surrounding water. These authors point out that oxygen consumption should be directly proportional to oxygen tension if there is an oxygen deficit in the tissues and if the rate of oxygen consumption is limited by the rate of diffusion of oxygen into the tissues.

Several investigators have found that in very small organisms in which diffusion of gases occurs very rapidly, oxygen consumption does not decrease appreciably with reduced oxygen concentration until a very low concentration has been reached. Below this critical pressure the oxygen concentration appears to limit the rate of functioning of the respiratory mechanism. SHOUP (41) has reviewed some of these studies and has shown experimentally that oxygen consumption by luminous bacteria is independent of oxygen pressure within the pressure range from 152 mm. of mercury (0.20 atm.) down to 22.8 mm. (0.03 atm.). Below the last mentioned pressure the curve for the rate of oxygen consumption as related to oxygen pressure is similar to curves for adsorption of gases at catalytic surfaces, which leads SHOUP to state that "luminous bacteria are so small that oxygen collecting at the catalytic surface of the oxidation mechanism of the cell becomes the limiting factor determining the rate of oxygen consumption rather than the oxygen diffusing into the cell." The respiratory activity of luminous bacteria ceased in pure nitrogen, and in pure oxygen the consumption of oxygen was irreversibly inhibited.

In the experiments on plant forms where no change in respiration was reported when the environmental oxygen pressure was increased or decreased, the periods of observation usually were short. JOHANNSEN'S measurements (19) of CO_2 production by sunflower seedlings successively exposed to air, air-hydrogen mixture and air, furnish evidence that the previous experience of the plants was an important condition in determining their respiration behavior in the short time periods employed. In the light of the studies of AMBERSON and his co-workers and of NOMURA, the

results secured by WURMSER and JACQUOT indicate that in the case of kelp, the rate of diffusion of oxygen into the tissues was a limiting factor in respiration on the supposition that the rate of diffusion would be directly proportional to the partial pressure of dissolved oxygen in the water. In the case of invertebrate animals differences in the responses of the several forms were probably determined by internal conditions of a regulatory nature.

If, under a given set of environmental conditions not dependent on oxygen supply, a plant or a plant tissue fails to show a changed rate of respiration corresponding to an alteration in the oxygen concentration of the surroundings, two possible explanations may be suggested. (1) The rate of oxygen supply within the cells may be proportional to the external partial pressure of oxygen but the rate of supply may not be a limiting condition under the circumstances; the lower rate of supply may be adequate, the higher rate producing neither increase nor decrease in respiratory activity. (2) An alteration of the partial pressure of oxygen in the environment may actually result in a change in respiration rate but there may be a marked time lag between the environmental change and the first appearance of the resulting internal effect, the observation interval being too short to show the response. The second suggestion emphasizes the extreme importance of duration in all physiological experimentation, a consideration that will be reverted to farther on.

INFLUENCE OF TEMPERATURE ON CO_2 PRODUCTION.—The literature concerning the influence of temperature on CO_2 production by plants was reviewed by KANITZ (**20**) in 1915, whose review included other life-processes of plants and animals, and by KOSTYTSCHEW (**25**, p. 16) in 1924. KANITZ attempted to analyze the relations of plant respiration and temperature in terms of temperature coefficients and pointed out that in many cases the quotient (Q_{10}) of the rate of CO_2 production for a given temperature divided by the corresponding rate for a temperature 10 degrees lower lies between 2 and 3 for some 10-degree ranges. The coefficient is lower, however, for 10-degree ranges in higher regions of the thermometer scale and higher for 10-degree ranges in lower regions of the scale. As FAWCETT has pointed out (**11**), the value of the 10-degree temperature coefficient for any process that has a temperature minimum and a temperature maximum must vary all the way from infinity (for 10-degree ranges partly below the minimum) to zero (for 10-degree ranges partly above the maximum), and it is not particularly important that the coefficient value is about 2 or 3 for some intermediate ranges. The interesting thing about temperature coefficients in general is not the actual value of the coefficient for any given 10-degree range but the manner of variation of the coefficient with regard

to the position of the 10-degree range in the temperature interval between minimum and maximum.

KOSTYTSCHEW emphasized the point that there appears to be no optimum temperature for the production of CO_2, above which the range is lower with higher temperature, for the rate gradually increases with rising temperature, to a maximum value at which it remains with still higher temperatures, until the thermal death point of the plant is reached. The same author referred also to the stimulating effect of abrupt temperature changes, as indicated by PALLADIN'S work on this subject (**33**). In PALLADIN'S studies, as cited by KOSTYTSCHEW, etiolated terminal buds of Windsor bean (*Vicia faba*) were kept on 10-per cent. sugar solution at low, medium and high temperatures for 3 days. When the rate of CO_2 production was then determined *at the medium temperature,* the rate was greater for the buds that had been kept at the high and at the low temperatures than for those which had been kept at the medium temperature and this had experienced no temperature change just prior to the measurement.

But KOSTYTSCHEW did not mention BLANC'S objections to PALLADIN'S interpretation. BLANC (**5**) pointed out that plant material kept for a time at one temperature is not to be considered as similar internally to material that has been kept for the same time at another temperature. In his own experiments, on etiolated terminal buds of Windsor bean (*Vicia faba*), young leaves of barley (*Secale cereale*) and embryos of ordinary bean (*Phaseolus vulgaris*), he ascertained the rate of CO_2 production for a given medium temperature, then exposed the material for an hour to a lower or higher temperature, and then again ascertained its rate of CO_2 production at the original medium temperature. The results showed a somewhat lower or somewhat higher rate in the second period at the medium temperature, according to whether the intermediate temperature was lower or higher, respectively, than the medium temperature. These observations were interpreted as failing to show stimulating effects for changes of temperature, although they indicate an after-effect of the intermediate exposure. It seems that the distinction among after-effects, lags, stimulations, etc., are very difficult and involved, and it may be best not to attempt any close analysis of the problem here suggested until much more experimentation has been carried out, with due attention given to all the influential conditions.

INFLUENCE OF CO_2 CONCENTRATION ON RESPIRATION.—SPOEHR and MCGEE (**42**) found that changes in the CO_2 content of the air surrounding a leaf had a temporary inverse effect on the rate of CO_2 emission. This effect varied in degree with different species. If the CO_2 concentration were maintained at a constant value after a change, the rate of CO_2 emission

finally became about the same as it was before the change. KIDD (**21**) found that both anaerobic and aerobic CO_2 production were reduced by CO_2 in the surrounding air. When oxygen was present, but in deficient amounts, so that some degree of anaerobic CO_2 production occurred, CO_2 in the atmosphere exerted no retarding effect. In ordinary aerobic respiration, with ample oxygen supply, however, the same quantitative relation was found between aerobic CO_2 production and the CO_2 concentration as between anaerobic respiration and CO_2 concentration. The inhibiting effect of CO_2 in the surroundings on germination of seeds, sprouting of tubers, and ripening of fruits, as found by KIDD and WEST (**22, 23, 24**), seems to be associated with its effects on respiration. The magnitude of the retarding influence was influenced by the concentration of oxygen in the surroundings.

INFLUENCE OF UNUSUAL CHEMICAL SUBSTANCES ON CO_2 PRODUCTION AND ON RIPENING PROCESSES.—Increased knowledge of the conditions that influence respiration promises to be valuable, and indeed quite essential, to an understanding of many forms of chemical stimulation, as, for example, the influence of ethylene on the ripening, blanching, etc., of fruits and vegetables, which has recently attracted much attention. The conflicting nature of the results reported by those who have made careful studies on the influence of ethylene suggests that this practical application can become understood and standardized only in terms of the basic physiological processes and rate changes that are involved, notably the phenomena of respiration. Some of the methods employed in treating plant material with this gas surely produce changes in the partial pressure of oxygen and of CO_2 about the stimulated tissues; for treatment in many instances has consisted in enclosing in a tight container the material to be treated, without any continuous flow of the gas mixture through the confined space. The influence of ethylene, or of any other gas, cannot be satisfactorily studied unless the method of continuous gas flow is employed. With suitable continuous flow of a known gas mixture the partial pressure of the gases used may be maintained almost constant and CO_2, or other gases produced, may be constantly removed, avoiding troublesome accumulation. An experimental study on the influence of ethylene upon respiration and growth of young wheat seedlings was carried out in connection with the investigation reported in this paper, but the results obtained, together with some discussion of the general problem of ethylene influence, are reserved for another publication, as has been noted.

INFLUENCE OF OXYGEN PRESSURE ON GROWTH AND DEVELOPMENT.—Among the early investigators who studied the relation of oxygen to growth and development should be mentioned the Swedish chemist, SCHEELE (**38**) (who

discovered oxygen independently in 1771), HUMBOLDT (**17**), ROLLO (**36**), DE SAUSSURE (**37**) and DÖBEREINER (**10**). Their experiments dealt mostly with germination of seeds and growth of seedlings in nearly pure oxygen and in ordinary air. Germination was more rapid in oxygen than in ordinary air, but seedling growth was less vigorous in oxygen. DÖBEREINER studied the enlargement of seedlings in air with increased and with reduced total gas pressure. Barley plantlets grown in air with half the ordinary total atmospheric pressure were more spreading, softer, and somewhat shorter than others grown at the same time in air with a total pressure of 2 atmospheres. There was more guttation in the plantlets grown with low pressure and this was considered to indicate higher turgidity. The differences in growth were attributed to turgor differences.

This subject was studied more thoroughly in the quarter century between 1873 and 1900, by BÖHM, BERT, WIELER, JENTYS, JACCARD and SCHAIBLE. BÖHM (**6**) found that the growth of plants at the expense of reserve materials was slower in nearly pure oxygen at a pressure of 1 atmosphere than it was in ordinary air, but growth in oxygen was more rapid (and like that occurring in ordinary air) if the oxygen tension were reduced until it was equal to that of the oxygen in ordinary air, the reduction being accomplished either by reducing the total gas pressure or by diluting the oxygen with hydrogen. BERT (**3**) concluded that variations in either direction from the ordinary partial pressure of oxygen as it occurs in the air resulted in decreased growth, and he attributed these differences in growth rate to differences in oxygen tension.

These studies of BERT, WIELER, JENTYS and SCHAIBLE were summarized by PFEFFER (**35**), who came to the generalization that most aerobic plants fail to grow in air at ordinary atmospheric pressure when the oxygen content of the air is less than from about 0.1 to about 3 per cent. by volume. He interpreted the effects of considerable differences in the total pressure of the surrounding air as influences on turgor, thinking that suitably diminished pressure increased turgor and so tended to accelerate enlargement, while sufficiently increased pressure at first reduced turgor and tended to reduce enlargement, though later it might evoke apparently adaptive responses of an opposite character, which PFEFFER designated as counter-effects. As to the influence of partial pressure or percentage of oxygen in the air about the plant, PFEFFER concluded that sufficiently increased oxygen percentage in air, with the ordinary total pressure, generally retarded growth, an effect apparently due to the oxygen itself, acting as a chemical influence. He pointed out that the cardinal points (minimum, optimum, maximum) of partial oxygen pressure, are dependent upon other influential conditions, especially upon the internal ones that are related to phase or stage of development, previous experience of the plant, etc.

In none of the experiments considered above were nearly all of the important influential variables taken into account. With green plants in the presence of effective light and with adequate supply of CO_2 the partial pressure of oxygen may be supposed to be high, at least in the illuminated green parts, this partial pressure being largely independent of the oxygen conditions of the surrounding air. The partial pressure of oxygen must be much lower in green parts after a period in darkness, and generally lower than that of the surrounding air in any parts into which oxygen does not diffuse at an adequate rate, as in the deeper-lying tissues of succulent leaves, stems, fruits, etc. On the other hand, when the aerial parts of green plants are kept in darkness or in sufficiently weak light for long periods the phenomena of etiolation are likely to lead to great complications in any attempted analysis of the relations between environmental conditions and the rate of enlargement.

HEUMANN'S more recent studies (**16**) did not greatly add to our information on the direct action of oxygen with respect to growth, for the reasons just suggested. His conclusion is that the acceleration of growth brought about by reduced partial pressure of oxygen in the air is caused by oxygen-hunger in the plant. He supported this idea with the observation that the morphological response to low oxygen supply was an increased area of organs, such as leaves, through which the oxygen of the surrounding air penetrates to the plant interior. The effects observed are thus attributed to an oxygen influence on general metabolism rather than to a direct influence on growth itself.

HARRINGTON (**14**) noted that when wheat seedlings were in an atmosphere containing 36 per cent. of oxygen growth was nearly twice as rapid as when they were in ordinary air, while further increases in the partial pressure of oxygen resulted in slower growth. He did not mention that light was excluded but light influence through photosynthesis may be supposed to have been of small consequence in these experiments, since seedlings in the early stages of their growth are not dependent to any considerable degree on the products of their own photosynthetic process, which is not rapid in these early stages.

It is evident that the relations between oxygen supply and growth in higher plants are very difficult to evaluate. Although the problem of growth-oxygen relations may be simplified to some extent by the exclusion of light in the experiments, yet the complications arising from the occurrence of etiolation cannot be avoided if the tests are continued throughout extended periods of time. The special responses of etiolation may be rendered less important if short experiment periods are employed, or the experimentation may be confined to germinating seeds or other tissues and organs

that may be kept for long periods in darkness without special effects arising from the absence of light. In any event, the influence of oxygen supply or of the partial pressure of oxygen in the surroundings must be studied with reference to as many of the vital processes as possible if we hope to approach this general problem successfully. For first approximations the general metabolic processes may perhaps be considered as summed up in terms of the rate of respiratory output of CO_2, and growth may be superficially estimated in terms of enlargement and other easily observed features of morphological development. Whenever the time period of an experiment in this field is sufficiently long observations should surely be made on both growth and respiration if such observations are at all possible.

THE TIME RELATION OF RESPIRATION AND GROWTH PROCESSES.—The importance of the time factor in physiological experimentation and discussion is suggested by some of the considerations in the foregoing paragraphs. Whenever the influence of conditions determining the rate of a process is to be analyzed it is essential that time be taken into account not only with reference to the measurement of the process rates themselves but also in the quantitative measurements of the influential conditions. Some aspects of this principle have been emphasized by LEHENBAUER (**26**), FAWCETT (**11**), HAASIS (**13**) and others and it is coming to be generally appreciated. Its special bearing in the present study lies mainly in the fact that developing organisms usually alter as they develop, respiration and growth rates increasing or decreasing with the progress of development. The only way in which a rising or falling process rate may be studied is by means of several short test intervals each of which is so chosen as to represent a suitable portion of the longer period during which the acceleration or retardation occurs. If test periods are too long the corresponding mean rates may be misleading, which is also true if total experiment periods are too short. When a rate is found to be altering with time, under maintained external experimental conditions, it is desirable to employ conveniently short, generally successive, test periods and to continue the experiment either till the rate becomes relatively constant, till it passes through a critical stage at which the sign of its acceleration changes, or till its acceleration becomes constant. When a new set of external conditions is brought to bear the behavior of the organism is usually related for a while to both the old set of conditions and the new set and the only way by which such lagging influences may be studied is through the employment of sufficiently short observation intervals in the earlier portion of the experiment period.

The length of the whole experiment period and the lengths of the partial periods employed for the rate tests are surely very important in such experiments as are required in the field here considered, for the rate of CO_2 evolu-

tion from germinating seeds and young seedlings increases with time, even when all external influences are maintained. These time relations are most important when it is necessary to alter the external conditional complex at the beginning of an experiment, as when seedlings are produced under one set of environmental conditions and are then transferred to another set for an experiment.

The last statement implies a consideration that is sometimes expressed by saying that the behavior of a given organism is partly determined by its past experience and partly by its current environment. Past experience, whatever it may have been, has resulted in the present internal conditions of health, vigor, tone, etc., and present behavior must really be considered as wholly controlled by the combination of current internal and current external influences. But we may be allowed to use the expression past experience to imply present internal characteristics as these have been molded by the environment and the organism operating together in the past.

In experiments that involve a change in the environmental complex, whether from preliminary treatment to the experimental conditions or from one set of conditions to another in the course of the experiment, the resulting changes in physiological processes are usually not abrupt and occur more or less gradually after the change in environment. It is therefore very difficult, as has been said, to separate the results of current influences from those of past influences. The immediate need for this sort of analysis may be avoided if we can find means for adequately defining or describing our experimental material at the beginning of an experiment, being as sure as may be that all coordinate experiments start with material having the internal characteristics thus described.

The estimation of the relative importance of past experience, current environment, and apparently adaptive responses can be arrived at most readily by analysis of the progressive changes in process rates which occur after the lapse of different lengths of time subsequent to specific changes in the environmental complex. As an illustration, let the plant material be changed from one environment to a second, temperature and the partial pressure of oxygen being considerably lower in the second than in the first. Subsequent observations might show the effects of several kinds of response as indicated by the respiration rate: The rate might decrease because of the lower temperature or because of the lower oxygen pressure or because of both these changes acting together; the rate might increase because of one or both of the changes and a tendency toward increase or decrease might be masked by a more pronounced tendency in the opposite direction; a tendency toward gradual increase would generally be expected, because the plant material continues to develop; and various apparently adaptive re-

sponses or counter effects might influence the rate as time went on. It is evident, therefore, that the acceleration of the rate of any process would only rarely be constant, and that changes in acceleration occurring under different sets of conditions, and in different test intervals are valuable clues to the separate responses that really occur.

Experimental methods

GENERAL PLAN OF THE EXPERIMENTS.—The experiments described in this paper were carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, between October, 1928, and March, 1929. The writer greatly appreciates the facilities placed at his disposal by the Johns Hopkins University and also the leave of absence granted to him by the Pennsylvania State College, for the academic year 1928–29, through which these studies were made possible. The writer is glad to express also his personal gratitude to Dr. BURTON E. LIVINGSTON, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for sincere interest, for valuable suggestions in the planning of the experiments, and for kindly criticisms on the preparation of this report.

These experiments were designed to take into consideration the difficulties brought out in the preceding introductory discussion. The process rates studied (CO_2 production and growth) were considered as the resultants of the combined action of the internal and environmental complexes of influential conditions. The influential conditions were considered as of two categories: (a) the background or parameter conditions, which were not consciously varied from experiment to experiment, and (b) the experimental variables (temperature and partial pressure of oxygen) which were maintained constant throughout a given experiment or any repetition of it, but differed from one experiment to another. The background conditions included (1) the internal conditions of the seedlings, which were as nearly alike as possible at the beginning for all experiments, being determined by the nature of the seeds and the preliminary treatment (germination procedure) to which they had been subjected; and (2) all external influential conditions excepting the two experimental variables. The background environmental conditions were planned to be alike for all experiments, being maintained nearly constant throughout the period of each experiment. These comprised water supply, salt supply, and light, the last having a value of zero because the cultures were kept always in darkness except for occasional momentary exposures to weak diffuse light for observations. Other influences which were kept constant or nearly so were the length of the experiment period and the lengths of the successive observation or test intervals within this period. For the different consecutive test intervals, how-

ever, the time was really an experimental variable; for example, the seedlings were of course younger in the first test interval than in the second, and the respiration rates in these intervals therefore corresponded to seedlings of different ages and different stages of development.

The main features of the general plan may be summarized as follows. Seeds of a specified stock were germinated by a specified technique and the resulting seedlings were allowed to develop in cultures, for a definite experiment period (46 hours) following a short period for transfer (0.5 hour) and adjustment (1.5 hour). Each experiment consisted of a single culture of 100 seedlings submerged in 250 cc. of standard nutrient solution, exposed to a certain maintained temperature, with a certain partial pressure of oxygen in the oxygen-nitrogen mixture continuously passing through the solution in the culture flask. Five experiments were carried out at the same time, constituting an experiment series. All of the five cultures in any series had the same oxygen-nitrogen mixture, but each differed from the other four with respect to its maintained temperature. Thus the oxygen-nitrogen mixture was the same for all experiments of the same series or repetitions thereof but differed for different series, while any given maintained temperature was employed for only one of the five experiments in any series. Each experiment thus differed from the others with respect to its combination of maintained temperature and oxygen pressure. Beginning at the end of the adjustment period (1.5 hour) the rate of CO_2 production was ascertained for five consecutive test intervals of 4, 6, 12, 12, and 12 hours, respectively, and observations on seedling development were made at the ends of the intervals, especially at the end of the fifth interval, which terminated the experiment in each case. The results show hourly respiration rates (as indicated by CO_2 production) for the several consecutive test intervals of each experiment and also notes on the corresponding growth rates for each experiment and each set of results corresponds to the specified oxygen-temperature complex that prevailed in the experiment from which they were derived, acting in connection with the internal and external background conditions previously mentioned.

THE WHEAT SEEDLINGS.—The wheat seed used in these experiments was of the Nittany variety, a pure-line winter wheat selected by Dr. C. F. NOLL, of the Pennsylvania Agricultural Experiment Station. The stock used was of the 1928 crop and was secured from the originator. It was stored in an aerated container at a temperature of about 20°–25°. The experimental lots, selected to include only sound, well-filled grains, showed a germination percentage of about 98 per cent., under the germination conditions provided. Twice as many seeds were germinated for each series of experiments as were required for the five cultures in the series, in order to allow for selection of

seedlings within relatively narrow limits. Thus 1,000 seeds were used for each experiment series, and five cultures of 100 seedlings each were regularly set up at the same time.

The method of germination, which was adopted after some preliminary tests, consisted in placing the thousand selected seeds in 500 cc. of distilled water in a 600-cc. Pyrex Erlenmeyer flask, and aspirating ordinary air through the water rapidly enough to cause a little movement of the seeds throughout the germination period, during which the flask was kept in the 20-degree compartment of the constant temperature apparatus. Temperature, moisture supply, and aeration were thus very nearly constant throughout the germination period and were practically the same for successive lots of seeds. Using the same quantity of water and the same temperature for each lot of 1,000 seeds gave assurance that changes brought about in the water by the soaking and germinating seeds (such as the outward diffusion of materials, which conceivably might influence germination) were of the same kind and occurred at about the same rates in all cases. No attempt was made to control the chemical content of the air aspirated through the water about the seeds; all the work was done in a well ventilated greenhouse in which plants were growing and no poisonous or stimulating gases could have been present in the air in significant amounts.

The length of the germination period was 42 hours, generally with a deviation of not more than a few minutes. In no case was the deviation more than 40 minutes. The temperature chosen for germination, actually about 19.5°, which is near the optimum for germination of wheat, lies at the middle of the range of the maintained temperatures used in the experiments. The mean temperature of the 20-degree chamber was a little below 20°, for there was a slight cooling at night. The successive lots of seedlings were as nearly alike as could be ascertained by ordinary observation, but of course there was considerable observable variability within each lot of 1,000 seedlings; in spite of the preliminary selection the seeds of a lot did not all reach exactly the same stage of germination in the 42-hour period. Nevertheless, the high degree of uniformity of the seeds and the uniformity of the germination treatment made it possible in every case to select the requisite five lots of 100 seedlings each, so that the populations of all cultures throughout the study, whether of the same or of different series, were apparently alike. The limits of development between which seedlings were selected were such that the coleoptile was always exposed, and the rootlets had not yet broken thorugh the coleorhiza.

It was of course conceivable that the lots of seed used for the later series of experiments might have differed constitutionally in some respects from the lots used for the earlier series, for the seed of the later lots was about

4 months older than that of the earlier ones and significant internal changes might possibly have occurred in the storage period. Some of the earlier experiments were repeated near the close of the work and the results of these showed clearly that the stock of seed had not altered significantly, as will be shown farther on when the experimental data are presented.

The standard conditions for germination constituted the environmental complex for the first 42 hours of soaking and germination, from which the subsequent experimental complex differed with respect to temperature, oxygen concentration and salt environment. Only in those particular experiments in which the maintained temperature was 20° and the partial pressure of oxygen was 20 per cent., was the oxygen-temperature complex the same for the experiment period as for the germination. In all other experiments, the seedlings experienced an abrupt lowering or raising either of oxygen pressure or temperature or both, at the beginning of the experiment. The CO^2 pressure in the environment was nearly constant throughout both the germination and experiment periods, CO_2 being removed by the constantly flowing gas stream in both cases, about as rapidly as it was formed. Since the seeds were germinated in distilled water and were in a standard weak nutrient solution for the experiments, all seedlings experienced a change in salt concentration and osmotic relations at the time the experiments were set up. This change, however, was the same for all experiments. The subsequent physiological activities of the seedlings through absorption and excretion, surely altered the solution during the progress of an experiment and the kind and degree of alteration may have been different for different experiments. Some of the experimental data indicate that these sources of discrepancy had no significant effect on the rate of CO_2 production, from the standpoint of this study. Nevertheless it would have been theoretically better if the same standard nutrient solution had been used for germination as for the subsequent experimental cultures, and if a constant flow of nutrient solution had been arranged as well as of the gas mixture. (On the theoretical advantages of continuously flowing solutions for solution cultures see TRELEASE (**45**) and SHIVE (**40**). No technique has been developed, however, for maintaining a constant flow both of gas and of solution through a culture from which CO_2 is to be removed and measured as it is produced.

THE MAINTAINED TEMPERATURES USED AND THEIR CONTROL.—The maintained temperatures tested were 10°, 15°, 20°, 25°, and 30° C. Preliminary tests showed that the experiment period and its consecutive observation intervals would need to be inconveniently long for temperatures below 10°, because of the slowness of growth and respiration at such low temperatures, especially for the earlier observation intervals. Consequently no temperatures below 10° were included in this study. Cultures at 30° showed but

little growth and there were some appearances of breakdown in seedlings held at that temperature two days or longer, while disintegration appeared in about 36 hours with a temperature of 40° and no growth was evident at that temperature. Since this study was not to deal with lethal and *post-mortem* phenomena, the range of temperatures tested did not extend beyond 30°. Temperature differences of 5 degrees were used partly because of convenience and partly because they were about as small as were warranted by the magnitudes of the temperature influences that were observed.

The battery of temperature chambers at the Hopkins Laboratory of Plant Physiology [LIVINGSTON and FAWCETT (**28**), HAASIS (**13**)] consists of a linear series of seven chambers with water jackets, separated from one another by uninsulated sheet-metal partitions, but insulated above, below and at the sides. The top of the apparatus is formed by the insulated wooden covers of the water baths. These are removable, but for convenience in manipulation, each is provided with three ½-inch holes, through which tubes leading to cultures, etc., may pass, or thermometers may be inserted, and also with a rectangular opening (23 x 28 cm.) closed by means of a removable insulated lid. Heat is supplied at one end of the series from a thermostatically controlled hot tank and removed at the other end by means of a cold tank thermostatically controlled by a mechanical refrigeration machine. Each bath is furnished with a mechanical stirrer, which slowly rotates in the water jacket around the cylindrical chamber, and all parts of each chamber are at practically the same temperature, but the temperature of each chamber differs from that of the adjacent chamber or chambers to a degree determined by the settings of the two thermostats. The temperature of the greenhouse in which the series of chambers stands was subject to fluctuations as great as 15° within a period of twelve hours or less and such changes in the temperature of the external air influenced the chamber temperature to some extent, in spite of the insulation, especially that of the chambers near the middle of the series, where the daily fluctuation, though ordinarily less than 1.0° was occasionally as great as 2.0°. The temperature of the middle chamber (20°) was usually from 0.5 to 1.0° too low for about 8 hours each night, and the average temperature of that chamber was really about 19.5°. Only five chambers were used in this study, the two end ones of the series of seven being idle. The chambers used were operated to give maintained temperatures close to 10°, 15°, 20°, 25° and 30°, as has been indicated.

Temperature records were made in the 10-, 20-, and 30-degree chambers, by means of small Richard thermographs, which were kept there throughout the experiments. The temperatures of the other two chambers were estimated by interpolation, with occasional direct observations by means of ordinary thermometers in these chambers.

THE GAS CONTROL.—The desired partial pressures of oxygen were secured by mixing compressed commercial oxygen and commercial nitrogen in a gas cylinder such as commercial gases are supplied in, the capacity of the cylinder being well above 2,000 pounds per square inch. A given mixture was prepared by connecting charged cylinders of nitrogen and oxygen successively to a previously discharged cylinder, by means of a copper tube (about 75 cm. long) provided with a suitable coupling at either end and with an ordinary pressure gauge closing a T-outlet in the middle. Each gas was allowed to flow into the mixing cylinder until the gauge gave the required reading, the proportions being measured in terms of pressure. A mixture of 10 volumes of oxygen and 90 volumes of nitrogen was secured, for example, as follows: Oxygen was first allowed to enter the mixing cylinder till the gauge showed a pressure of 100 lbs., after which the valve of the mixing cylinder was closed and the oxygen supply tank was removed. Then nitrogen was allowed to flow into the mixing cylinder till the gauge showed a pressure of 1,000 lbs., after which the valve was again closed and the connecting tube was removed. The result was a tank of gas mixture of practically the required proportions and with a pressure of 1,000 lbs. per square inch. A tank of gas mixture might be kept closed till needed, when it was connected to the gas line leading to the culture flasks.

The compositions of the commercial gases and of the mixtures containing less than 30 per cent. of oxygen were ascertained through absorption of the oxygen by phosphorus. In the analysis of commercial oxygen a small amount of the gas was introduced into the eudiometer and its volume was measured, after which commercial nitrogen (from which the oxygen had previously been absorbed by means of phosphorus) was added in quantity sufficient to reduce the percentage of oxygen to less than 50 per cent. The mixture thus formed was then analyzed and the necessary computations were made.

The gas mixtures were delivered into the tubing lines leading to the culture flasks at a pressure regulated by means of a Hoke Phoenix Regulator or pressure-reducing valve. The maintained rate of flow through a flask was determined by the pressure of the supply and by an orifice control in each tube. Before reaching the separate lines leading to the cultures, the gas was passed first through a tube (of 18-mm. bore and 30 cm. long) containing soda lime, to remove any CO_2 which might be present, and then through concentrated H_2SO_4, to remove moisture, which might have tended to clog the orifice controls. The gas was then delivered to the separate lines through a 5-branched manifold of copper tubing.

The orifice controls were similar in principle to those described by HUTCHINS (**18**). Each consisted of a section of glass tube (of 5-mm. bore

and 12 cm. long) with a slight constriction about 3 cm. from one end and a plug of alternate layers of dry cotton and powdered chalk packed in the longer portion, against the constriction. The gas passed through the plug before reaching the constriction and the pressure of the gas thus tended to hold the plugs firmly in position. Alternate layers of cotton and chalk were found to be more easily adjusted than a single mass of kaolin or chalk between two cotton plugs, which was employed by HUTCHINS. The controls were adjusted so that the gas flow was 3 cc. per minute for each pound of pressure registered on the gauge of the pressure-reducing valve. The rate of gas flow (measured by means of a eudiometer) was found to be directly proportional to the pressure with this arrangement and it could therefore be controlled at will by setting the valve for the proper delivery pressure. The orifice controls required one or two slight adjustments in the first few days of use, perhaps because of the drying of the cotton or chalk. After these preliminary adjustments, the standard ratio of flow rate to gauge reading remained constant as long as the controls were in use, as was shown by occasional tests.

Between the orifice control and culture flask the gas was led through a telltale, which consisted of a rubber-stoppered bottle with inlet and outlet tubes arranged so that the gas bubbled through about 50 cc. of very dilute barium hydroxide solution colored with a few drops of phenolphthalein. Each telltale stood alongside the culture flask it served, in the same temperature chamber, and the incoming gas was here brought to the temperature of the culture chamber before entering the flask. The constant pink color of the solution indicated the absence of CO_2 in the gas stream. Also, moisture was added to the gas here, so that the volume of the nutrient solution in the culture flasks was not significantly altered by evaporation or condensation.

The total gas pressure above the solution in the culture flasks was always slightly higher than the prevailing atmospheric pressure, because of the hydrostatic pressure of the solution in the absorption-tower and in the telltales, but this difference was considered as negligible, especially since the pressures were the same for all cultures in a given series of experiments. The partial pressure of CO_2 in the culture flasks was kept practically at zero, by sweeping out the CO_2 as rapidly as it was formed.

The rate of gas flow through the culture flasks was established at 20 cc. a minute, after a number of preliminary experiments on the effect of variation in the rate of gas flow. In these preliminary experiments a gas mixture high in oxygen was used, and the rate of flow was different for successive experiment series, with differences of 5 cc. or less for a range from 5 cc. a minute to 20 cc. The gas mixture was presumed to be nearly pure oxygen,

but was not analyzed. It was subsequently indicated, however, to contain about 85 per cent. of oxygen by comparison of results with it and with mixtures the composition of which was known. Experiments were performed also with a mixture containing approximately 20 per cent. of oxygen, some with a rate of gas flow of 5 cc. a minute and some with a flow of 20 cc. a minute. In the experiments with a high percentage of oxygen, the rate of CO_2 production was greater for increased rates of flow up to 15 cc. a minute, but showed no significant changes for higher rates. Growth at the higher temperatures also increased as the rate of gas flow was raised, up to 15 cc. a minute, but did not change for rates greater than this. The proportional increase in CO_2 production for an increase in rate of gas flow from 5 to 20 cc. a minute was found to be practically the same for seedlings in 20 per cent. of oxygen as it was for seedlings in the high percentage of oxygen. This increase in CO_2 production for greater rates of gas flow was probably due to more thorough mixing of the nutrient solution, which resulted in a more uniform supply of oxygen to all the seedlings and more rapid removal of CO_2 from them. The lowest rate of gas flow supplied oxygen to the culture at a rate far in excess of oxygen consumption by the seedlings, as indicated by their CO_2 output. The higher rate of gas flow resulted in fairly rapid convection throughout the nutrient solution; a droplet of India ink became evenly dispersed throughout the solution in less than a minute when the gas flow was 20 cc. a minute.

The rate of gas flow therefore was established at 20 cc. a minute, since this rate was evidently more than sufficient to maintain CO_2 production and growth at maximum rates, as far as oxygen supply was concerned, for all the temperature-oxygen combinations tested.

THE CULTURE FLASKS.—The culture flasks were of Pyrex glass, of the Erlenmeyer form, with capacity of 300 cc. Two sets of five were used, so that one set could be cleaned and prepared for an experiment while the other five were still in use. Each flask had a tightly fitting 2-hole rubber stopper through which the inlet and outlet tubes were led. Because flexibility was desirable these tubes were of lead, but the metal did not come in contact with the culture solution at any time. The lead portion extended only about 15 mm. below the stopper, and the inlet tube was extended by a glass portion, attached by an ordinary rubber-tubing coupling, nearly to the bottom of the flask. The entering gas mixture bubbled through the culture solution, and passed out through the other tube. The outlet tube led directly to its own absorption apparatus.

To avoid any possibility of leakage the stoppers of the culture flasks (and also the stoppers of the telltale bottles) were secured by means of special clamps. A horizontal triangle of $\frac{1}{8}$-inch brass plate with an opening in its

center (to accommodate the inlet and outlet tubes) rested on the top of the rubber stopper and was connected with a base piece by means of three vertical 3/16-inch brass rods threaded at either end and provided with brass nuts (from discarded electric dry cells). The base piece on which the flask stood was a horizontal hexagon (about 2.5 cm. thick) of 2-ply wood with three holes near its edge, for the vertical rods. When the clamp was in place and the nuts were tightened the two plates were drawn toward each other and the stopper was held firmly in position in the neck of the flask.

TUBING AND CONNECTIONS.—Most of the tubing lines were of glass, with wired couplings of thick-walled rubber tubing, the joined ends being in contact. Where flexibility was required lead tubing was employed, it being joined to the glass sections by wired couplings of rubber. There were no joints between the orifice control and the absorption apparatus excepting those formed by the rubber stoppers of the culture flask and the entrance telltale, which were very tight. All lead tubing used was first tested for pin-holes, etc., by applying a gas pressure of 20 pounds per square inch with the tubing under water. The tubes for liquid were of glass or flexible rubber.

THE ABSORPTION APPARATUS.—The absorption apparatus embodied some of the principles of an apparatus described by CARRICK (8), with modifications to allow titrations to be made within the apparatus. The details of construction are shown by the diagram of figure 1. The essential features are: the absorption tower, A; the standard acid burette, B; the titration bottle, G; the vent, E, guarded with the soda-lime tube D; the siphon outlet, I; the inlet for standard alkali solution (or water for washing), J; the outlet telltale, M; an additional vent, K, and the outlet, L, both guarded with soda-lime tubes; and the gas inlet tube, F, connecting the apparatus with the culture flask, from which it is the outlet tube. A complete absorption apparatus was provided for each of the five culture flasks, being permanently mounted on a shelf at the side of the constant temperature chamber in which the culture flask was located.

The absorption tower (A, fig. 1) consisted of a heavy-walled glass tube (18 mm. in inside diameter and 55 cm. long) containing a column of glass beads (3–5 mm. in diameter) about 35 cm. high, the beads resting on an inverted glass vial (C) about 7 cm. high, with 5 or 6 holes blown in its bottom. Both vial and beads were found to be required for complete absorption of the CO_2 brought from the culture flask in the gas stream. The inverted vial provided a reservoir of absorbing solution, into which the entering gas bubbles were discharged and in which they were held for a short period, until succeeding bubbles caused the trapped gas to move upward through the holes and among the beads.

The outlet telltale (M, fig. 1) was simply a test tube (20 x 150 mm.) provided with a 2-hole rubber stopper and inlet and outlet tubes, so arranged that the gas escaping from the absorption tower bubbled through a measured quantity of standard barium hydroxide solution before being discharged. The outlet of the telltale was of course guarded by means of a soda-lime tube. Absence of any white precipitate, in this telltale, which was in plain sight, made it certain that no CO_2 had found its way through the absorbing tower.

When absorption of CO_2 was in progress, all of the three rubber connections marked X on the diagram (fig. 1) were closed with Hoffman clamps. A definite quantity of standard barium hydroxide solution (0.2 normal) was measured from a charging burette into the absorption tower through the solution inlet (J, fig. 1) and was washed down with a small quantity of CO_2-free water. The pressure of the gas entering through the gas inlet (F, fig. 1) held the alkali solution in the tower and the gas bubbles rose through this solution, being delayed and broken up by the vial (C) and the glass beads. Thence the gas stream was conducted through the outlet telltale (M) and was finally discharged through the discharge outlet (L).

When a determination was to be made, at the end of a test interval, the vent (K) at the top of the absorption tower was opened first. A few drops of phenolphthalein solution was introduced through the solution inlet (J) and a tube leading from a reservoir of distilled water above the apparatus was then connected to the solution inlet. The vent (E) on the titration bottle was then opened and the solution in the tower was drained into the titration bottle (G) through the tube H. Water was then allowed to flow in through the solution inlet (J) until all traces of pink color had been washed down. The excess of alkali was then determined by titration with standard HCl solution (0.1 normal), from the burette B. The connections were sufficiently flexible to allow shaking of the titration bottle (G), during the titration. When the end-point was reached the neutralized solution was allowed to flow out through the discharge siphon (I), by opening the clamp IX. The siphon tube itself was not emptied, since the water in it provided an insurance against leakage past the clamp. Tower and bottle were finally washed with distilled water, and a new charge of standard alkali solution was introduced as at the beginning, all clamps being properly adjusted.

THE STANDARD ACID SOLUTION.—The standard acid solution used for titration and for standardizing the standard alkali solution was approximately 0.1 normal (0.1132 N) HCl. This was standardized originally against dry sodium carbonate, with methyl orange as indicator, and the standardization was repeated from time to time during the progress of the experimental work, to ascertain whether changes occurred in the concentra-

tion of the acid under the conditions of storage. Eighteen liters of the standard acid solution were prepared at the beginning of the work, and this was sufficient for the entire series of experiments. This stock was kept in an 18-liter bottle closed with a 2-hole rubber stopper carrying a glass siphon for withdrawal of solution and an air inlet, the latter protected by a soda-lime tube to exclude CO_2. Two 4-liter reservoir bottles were filled from this stock, and were kept on an overhead shelf suspended from the frame of the greenhouse. Standard acid solution was delivered to the acid burette (B, fig. 1) of each absorption apparatus by means of glass tubing with rubber connections for flexibility. The reservoir bottles were stoppered in the same way as was the large stock bottle.

THE STANDARD ALKALI SOLUTION.—The standard barium hydroxide solution was prepared by dissolving "Baker's Analyzed" barium hydrate crystals in distilled water at the rate of 31.554 gm. per liter of solution with addition of 4.5 gm. "Baker's Analyzed" barium chloride per liter. The latter was added to reduce the solution and hydrolysis of the $BaCO_3$ which was present at the time titrations were made. An 18-liter stock bottle of this alkali solution was prepared at the beginning of the experiments, and this was sufficient for all of the work. The bottle was closed like the stock bottle for the standard acid solution. The small amount of barium carbonate present (there is usually some carbonate in barium hydroxide) was first allowed to settle and then the clear solution was siphoned into reservoir bottles like those used for the standard acid solution and similarly placed. From these reservoirs the solution was delivered to the charging burettes by means of a branching system of glass tubing with short rubber connections for necessary flexibility, as shown by the diagram in figure 2, described below. All air inlets into the distributing system for standard alkali solution were provided with soda-lime tubes, to prevent the entrance of atmospheric CO_2. That no CO_2 entered the system was shown by repeated tests of the solution as delivered by the burettes, titrations being made with standard acid solution and phenolphthalein.

CHARGING THE ABSORPTION APPARATUS.—The charging burettes were arranged to receive and deliver standard alkali solution without its coming into contact with atmospheric CO_2. Their arrangement is shown in figure 2. The supply tube (T) leading from the reservoir bottle (R) is connected to the recurved tube (C) of an automatic burette (B) through the 2-way stopcock (CA). Solution flows into the burette when the cock is in the position shown and when the cock is turned half-way around solution is discharged through the tip A. Air is allowed to escape from or enter the burette through the opening above, which is fitted with a 1-hole rubber stopper bearing a soda-lime tube (G).

When an absorption tower was to be charged with alkali solution a small amount of solution was first allowed to run out of the burette into a beaker, to remove what had been in the tip (A), in contact with the air. The burette was then lowered slightly and the tip was connected immediately with the solution inlet of the absorption tower (J), after which the desired amount of solution was allowed to run into the tower. After closing the 2-way cock and disconnecting and raising the burette a small amount of CO_2-free water was introduced into the solution inlet before this was closed, to carry down all the solution measured from the burette. It was found that the possibility of error was reduced if the excess of standard alkali solution did not greatly exceed the equivalent of 3 or 4 cc. of the standard acid solution.

Repeated checks were made in the following manner, to test the accuracy of the method of titration within the apparatus. Some of the $BaCO_3$ from a previous experimental test was allowed to remain in the titration bottle (G, fig. 1), after the excess alkali had been neutralized and most of the liquid had been siphoned out. A measured quantity of standard alkali solution was then introduced into the absorption tower in the manner described above, and was washed down into the titration bottle with distilled water after a few drops of phenolphthalein had been added. The water for washing was introduced into the top of the tower by connecting a tube leading from a reservoir of water on the shelf above the apparatus, to the solution inlet (J, fig. 1). Ordinarily a total of 150 cc. of water, introduced in several portions, was sufficient to remove all traces of alkali from the tower, as indicated by the disappearance of pink color from the beads in the tower. The alkali solution thus introduced into the titration bottle was titrated immediately with the standard acid solution. The results of these titrations in the apparatus, in the presence of the $BaCO_3$ mentioned above, agreed with other results obtained with a similar quantity of the standard alkali solution, taken directly from the charging burette (B, figure 2) into a flask containing 150 cc. of distilled water, and titrating immediately with standard acid solution from a burette separate from the apparatus, using phenolphthalein as an indicator. This agreement of results indicates that $BaCO_3$ is not a source of error in this sort of titration, [which is in agreement with statements by TREADWELL and HALL (**44**, p. 485) and BERGMAN (**2**).] These repeated tests, both inside and outside the apparatus, served also as checks on the constancy of the standard alkali solution.

TITRATION.—As is evident from what has been said, titration was accomplished in the presence of the $BaCO_3$ that had been formed by the reaction of the absorbed CO_2 and the $Ba(OH)_2$ of the alkali solution in the absorp-

tion tower. At the end of a test interval, the solution in the tower was washed down into the titration bottle (G, figure 1), in the manner previously described. Very nearly the same amount of distilled water was used in each case. The water for washing was guarded against contact with atmospheric CO_2 by a soda-lime tube in the air inlet of the water-reservoir bottle.

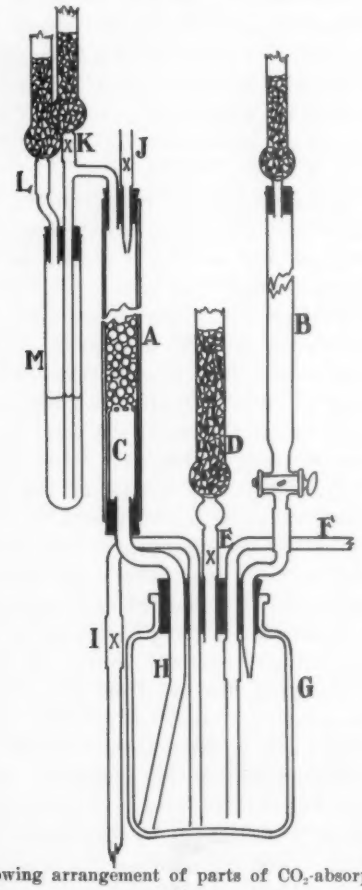

FIG. 1. Diagram showing arrangement of parts of CO_2-absorption apparatus. For description see text.

Standard acid solution was added drop by drop, with constant shaking of the titration bottle, to avoid any local excess of acid, which might react with the $BaCO_3$. During the process of washing down and titrating, which

required about five minutes, the gas stream continued bubbling through the solution in the titration bottle. Consequently the only loss of CO_2 which occurred took place while the neutralized solution was being discharged and the apparatus was being washed, preparatory to the addition of a new charge of alkali solution. This slight loss was obviously insignificant.

THE CULTURES.—Each culture consisted of 100 seedlings in 250 cc. of standard nutrient solution contained in a 300-cc. flask. The standard nutrient solution was SHIVE's solution R5C2 (**39**), calculated to have an

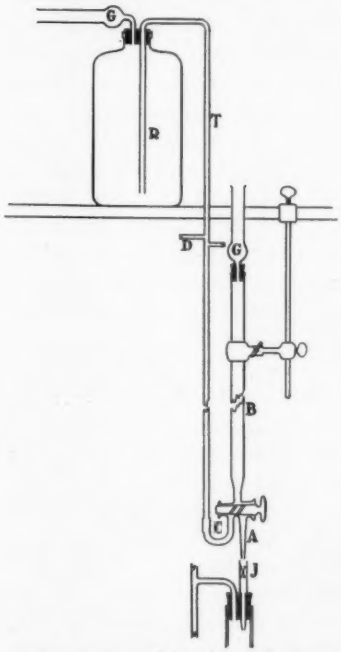

FIG. 2. Diagram showing arrangement of burette and reservoir for charging the CO_2-absorption apparatus. For description see text.

osmotic value of 0.175 atmosphere. Each liter contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of KH_2PO_4 and 0.3698 gm. of $MgSO_4$; no iron was added. Eight liters of stock nutrient solution were prepared, 10 times as concentrated as was required. The salts used were from unopened bottles originally prepared in connection with the cooperative experiments planned by the Division of Biology and Agriculture of the National Research Council (**27**), by the Powers-Weightman-Rosengarten Co. The concentrated stock nutrient solution was prepared by dissolving the three salts together, to form

a measured volume of solution, rather than by mixing separate standardized single-salt solutions. No precipitate appeared.

When a set of five cultures was to be started the requisite volume of solution was made up by diluting 125 cc. of the stock solution to 1250 cc., 250 cc. of which was placed in each of the five culture flasks, which were numbered and stoppered and then distributed in the five temperature chambers, where each flask and its contents soon came to the temperature of the chamber. Then the 1,000 germinated seeds that had been for 42 hours in water in the germination flask in the middle chamber (19.5°) were spread out in a glass pan, from which they were transferred one by one (with bone-tipped forceps) to the culture flasks, which were removed from their chambers and remained at room temperature only for the 20 minutes required for selection and transfer. In order that the five 100-seedling lots might be as nearly alike as possible, that every lot might contain about the same proportions of somewhat larger, somewhat smaller seeds, etc. (although, as has been said, the 500 seedlings selected for a set of cultures were very nearly alike), the five lots were not selected consecutively; instead of that, 10 or 20 seedlings were transferred to one flask, then a like number to another flask and so on till every flask had received its 10 or 20 seedlings, after which this procedure was repeated in rotation, till every flask had its full quota of 100 seedlings. As soon as the transfer had been completed each flask was returned to the chamber in which it and its solution had previously been warmed or cooled, being immediately placed in connection with the gas line (with stopper set firmly in place and flask clamp tightened to insure a permanent seal) and the flow of gas mixture began.

At the close of a series of experiments (*i.e.*, immediately after the titration for the fifth test interval) the culture flasks were removed from the apparatus and their seedlings were examined with respect to the growth that had occurred. The seedlings were then discarded and the flasks were scrubbed with hot water, rinsed with distilled water and thoroughly dried, being then ready for another series of experiments. No special procedure was adopted for the sterilization of flasks and nutrient solution, nor were the wheat seeds disinfected before being placed in the germination flask. There was no evidence of growth of fungi or bacteria in any of the cultures, even at the higher temperatures.

Changes in the culture solution in the experiment period were ignored, as has been said. That such changes must have occurred, because of absorption and excretion, was indicated by a few brief studies with seedlings in nutrient solutions in which other seedlings had previously been grown for a 24-hour period, but these changes were found to be slight, probably because of the large volume of solution used for each culture and the short duration of the experiment period.

MEASUREMENTS OF CO_2 PRODUCTION.—Measurements of CO_2 production were begun 2 hours after each series of culture flasks received their seedlings, or approximately 1.5 hour after the cultures were placed under the experimental conditions. At the end of each test interval the total production of CO_2 for that interval (the amount of CO_2 that had been absorbed in the absorption apparatus during the interval) was ascertained by titration. About half an hour was required to make the five titrations, which were made always in the same order from the lowest to the highest temperature, so that the intervals were very nearly the same for the different experiments in a series. The deviation from the stated time of observation was usually less than 15 minutes; in a few cases, however, it was greater, sometimes as much as 45 minutes. The actual length of the time interval referred to by each titration record was recorded in every instance, however, and the mean hourly rate of CO_2 production was computed. These mean hourly rates for the five test intervals of each experiment are the numerical data on CO_2 production with which we shall deal when the results of these experiments are presented farther on.

OBSERVATIONS ON GROWTH.—At the end of each experiment, 48 hours after the seedlings had been placed in their experimental environment, the seedlings were removed from the culture solutions and measurements of growth were made. Although the roots elongated more rapidly than the shoots their measurement presented so many difficulties (especially because the number of roots per seedling was variable) that growth records were based on shoot elongation alone. Shoot elongation also was variable, especially at the higher temperatures, and many seedlings made so little growth in the experiment period that actual measurement was not practicable. After considerable study the following procedure was adopted for securing numerical indices of shoot elongation. All shoots that could be measured with satisfactory accuracy were measured to the nearest 0.2 millimeter, using a millimeter scale, and the average of the ten longest ones in an experiment was taken as the measure of the growth rate for that experiment. Of course these indices of growth rate are not precise, but they are sufficiently consistent to be useful, as will be seen later.

THE COMPUTATION OF AVERAGES.—The total amount of CO_2 collected during an interval was first expressed in terms of the equivalent volume of standard acid solution, in cubic centimeters, and this value was then multiplied by the standard factor 2.49, for 1 cc. of standard acid solution had been shown to be equivalent to 2.49 mg. of CO_2. The resulting product was the number of milligrams of CO_2 produced by the given culture in the given interval. This product was next divided by the length of the corresponding interval, expressed in hours, to give the mean hourly rate of CO_2 production for the interval. Five of these mean hourly rates were

thus secured for each experiment and 25 for each experiment series. In one or two instances where the number of seedlings per culture was not 100 the mean rates were all computed to represent 100 seedlings, which they did actually represent in most cases.

A preliminary, point-to-point graph was drawn to represent the five mean rates for each interval, as these differed from temperature to temperature in the same experiment series, one graph for the first interval, another for the second, etc. The ordinates of these preliminary graphs were the mean hourly rates of CO_2 production and the abscissas represented the corresponding temperatures, derived from thermograph or thermometer records. Each experiment series thus gave five graphs, one for each of the consecutive test intervals, and each group of graphs represented one of the 12 different oxygen-nitrogen mixtures used. Since the actual temperature means were not always exactly the standard maintained temperatures that they represented, the ordinates of each graph were corrected where necessary, to correspond more closely to the requisite temperatures, by simply measuring the actual ordinates for those temperatures (10°, 15°, 20°, 25°, and 30°) and employing the resulting values instead of the actual mean rates from which the graph had been constructed. By these procedures a mean hourly rate of CO_2 production by 100 seedlings was obtained for each of the five required temperatures and for each of the five test intervals in every experiment series, some slight discrepancies being thus avoided while all series were brought into conformity with regard to the five temperature values. Because every experiment series was repeated at lest once there were two or more mean hourly rates of CO_2 production for each of the 60 different combinations of temperature and oxygen pressure for each interval and these were finally averaged for each combination.

For the entire experiment period the total amounts of CO_2 produced with each combination of temperature and oxygen pressure were ascertained for each experiment, and these totals were finally averaged for each group of like experiments. Sixty average totals were thus secured, one for each combination of temperature and oxygen pressure used.

Results and discussion

General nature of the results

The main results of this study will now be presented, with notes on their interpretation. It is to be borne in mind that the conclusions reached are applicable only to the specific sets of internal and external conditions that prevailed in the tests. The data presented are to be studied to bring out any relations that may appear among five kinds of variables, always

with reference to the conditions of the internal and external background of the experiments, which have been described. These variables are: (1) maintained temperature, (2) partial pressure of oxygen, (3) relative stage in the progress of seedling development (indicated in each experiment by the interval number), (4) rate of CO_2 production and (5) rate of shoot elongation. Expressed in another way, the results are to be considered with reference to the manner in which the rates of CO_2 production and of shoot elongation differ, from interval to interval in the same experiment, and from experiment to experiment in relation to the several combinations of maintained temperature and maintained oxygen pressure. There are to be considered 12 different partial pressures of oxygen (the total gas pressure being always 1 atmosphere) for each of the five different maintained temperatures, and each of the 60 different combinations of temperature and oxygen pressure is to be considered with regard to each of the five consecutive test intervals of each experiment.

No evidence of aging of seeds

The experiment series with partial pressure of oxygen about like that of ordinary air (20 per cent.) was carried out seven times, as chronologically numbered series 1, 2, 3, 4, 23, 37 and 38. (Series 5–22 and 24–36 had other oxygen pressures.) The first four series were carried out between December 10 and December 18, 1928, series 23 was carried out in February, 1929, and the last two were carried out between March 20 and March 24, 1929. The actual partial pressures of oxygen for these series were: series 1, 2 and 3, 21.3 per cent.; series 4, 20.4 per cent.; series 23, 20.0 per cent.; series 37 and 38, 18.9 per cent.; but these differences are not large enough to be significant. A study of the results of these series (brought together in table I) fails to reveal any consistent suggestion that the seedlings of series 1 or 2 may have been initially different from those of series 37 or 38 and leads to the conclusion that the stock of seed used in this study did not alter significantly during the progress of the work. It is also clear from the value of table I that the standard germination technique was not significantly altered as the work went on. These are considerations of the utmost importance in studies of this kind.

CO_2 production in the several observation intervals and in the entire experiment period

The tabulated values.—The 60 average rates of CO_2 production for each of the five intervals are represented in table II. The five horizontal subdivisions of the table represent the five test intervals, which are numbered consecutively and designated in the following manner: 1st interval,

TABLE I

COMPARISON OF RESULTS FROM EXPERIMENT SERIES NEAR BEGINNING AND NEAR END OF STUDY, WITH OXYGEN PRESSURE ABOUT 20 PER CENT.

EXPERIMENT SERIES NO.	DATES OF EXPERIMENT	TEST INTERVAL	MEAN HOURLY RATE OF CO_2 PRODUCTION (100 SEEDLINGS) FOR TEMPERATURE OF				
			10° C.	15° C.	20° C.	25° C.	30° C.
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	Dec.	1	0.33	0.55	0.70	0.88	1.25
	12–14	2	0.34	0.62	0.77	1.14	1.88
	1928	3	0.36	0.77	1.07	1.37	2.13
		4	0.39	0.94	1.18	1.76	2.78
		5	0.46	1.12	1.50	2.26	3.63
2	Dec.	1		0.61	0.83	0.87	1.37
	14–16	2		0.60	0.85	1.15	1.93
	1928	3	0.37	0.76	1.03	1.35	2.38
		4	0.47	0.93	1.29	1.75	3.27
		5	0.49	1.08	1.69	2.34	3.82
3	Dec.	1	0.36	0.78	0.95	1.10	1.39
	16–18	2	0.42	0.77	0.93	1.40	1.78
	1928	3	0.52	0.83	1.09	1.59	2.47
		4	0.66	1.01	1.31	2.04	3.57
		5	0.73	1.19	1.58	2.72	4.50
4	Dec.	1	0.40	0.74	0.84	0.95	1.49
	18–20	2	0.39	0.72	0.87	1.08	1.81
	1928	3	0.51	0.84	1.06	1.42	2.37
		4	0.58	0.98	1.41	2.07	3.49
		5	0.68	1.22	1.69	2.49	4.03
23	Feb.	1	0.35	0.60	0.77	1.02	1.19
	12	2	0.42	0.62	0.78	1.12	1.63
	1929	3	0.45	0.73	1.02	1.61	2.32
37	Mar.	1	0.44	0.62	0.82	0.96	1.23
	20–22	2	0.46	0.62	0.81	1.07	1.64
	1929	3	0.48	0.71	1.04	1.40	2.14
		4	0.54	0.91	1.29	1.77	3.01
		5	0.63	1.22	1.71	2.33	4.00
38	Mar.	1	0.43	0.52	0.80	0.87	1.20
	22–24	2	0.41	0.52	0.89	1.18	1.64
	1929	3	0.47	0.73	1.16	1.47	2.14
		4	0.54	0.88	1.50	1.98	3.21
		5	0.68	1.09	1.87	2.51	

Note.—Experiment series 23 was continued for three intervals only. The two blanks for series 2 and one for series 38 indicate that the data were lost.

the four hours following the close of the adjustment period (*i.e.* the 3rd to the 6th hour, inclusive, after transfer of seedlings to culture flask); 2nd interval, the next 6 hours (7th to 12th hour of the experimental period); 3rd interval, the next 12 hours (13th to 24th hour); 4th interval, the next

TABLE II

TABLE II

AVERAGE RATES OF CO_2 PRODUCTION BY 100 SEEDLINGS FOR EACH TESTED COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN EACH OBSERVATION INTERVAL

INTERVALS	TEMPERATURE	AVERAGE MEAN HOURLY RATE OF CO_2 PRODUCTION WITH OXYGEN PRESSURE OF											
		0.6 PER CENT.	3.1 PER CENT.	6.3 PER CENT.	9.8 PER CENT.	16.0 PER CENT.	20.0 PER CENT.	30.0 PER CENT.	50.0 PER CENT.	75.0 PER CENT.	90.0 PER CENT.	95.0 PER CENT.	98.3 PER CENT.
	deg. C.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1ST	10	0.36	0.38	0.38	0.42	0.44	0.39	0.44	0.45	**0.47**	0.46	0.46	0.46
TEST	15	0.46	0.55	0.61	0.56	0.58	0.63	0.71	0.74	0.82	**0.84**	0.75	0.71
INTERVAL	20	0.63	0.73	0.73	0.86	0.76	0.82	0.83	0.94	1.10	**1.17**	1.02	0.98
(3RD TO	25	0.74	0.88	0.93	1.02	0.97	0.95	1.05	1.20	1.38	**1.50**	1.29	1.23
6TH HOUR,	30	0.96	1.03	1.16	1.21	1.23	1.30	1.34	1.60	1.91	**2.08**	1.87	1.55
INCLUSIVE)													
2ND	10	0.26	0.33	0.31	0.34	0.41	0.41	0.54	0.56	0.59	**0.64**	0.52	0.53
TEST	15	0.41	0.52	0.56	0.54	0.56	0.64	0.72	0.85	0.98	**1.04**	0.93	0.92
INTERVAL	20	0.61	0.73	0.77	0.80	0.83	0.84	0.94	1.05	1.37	**1.57**	1.38	1.36
(7TH TO	25	0.79	1.00	1.14	1.37	1.20	1.16	1.24	1.44	1.81	**2.04**	1.74	1.80
12TH HOUR,	30	1.11	1.32	1.72	1.86	1.75	1.76	1.69	2.02	2.56	**2.88**	2.55	2.42
INCLUSIVE)													
3RD	10	0.27	0.30	0.34	0.33	0.44	0.45	0.59	0.69	**0.75**	0.73	0.66	0.63
TEST	15	0.46	0.62	0.61	0.52	0.62	0.78	0.83	0.97	1.30	1.42	**1.55**	1.25
INTERVAL	20	0.66	1.00	1.11	1.06	1.08	1.07	1.21	1.43	1.98	**2.41**	2.20	2.24
(13TH TO	25	0.93	1.52	1.81	1.95	1.74	1.46	1.67	1.93	2.80	**3.32**	3.16	3.18
24TH HOUR,	30	1.38	1.97	2.29	2.36	2.62	2.28	2.25	2.88	3.88	**4.65**	4.54	4.07
INCLUSIVE)													
4TH	10	0.28	0.35	0.37	0.36	0.51	0.53	0.70	0.86	0.82	**0.89**	0.76	0.77
TEST	15	0.47	0.88	0.82	0.70	0.75	0.94	1.04	1.25	1.66	1.91	**2.04**	1.52
INTERVAL	20	0.70	1.30	1.37	1.18	1.17	1.33	1.46	1.88	2.82	3.49	**3.60**	3.54
(25TH TO	25	1.03	1.85	2.06	2.31	2.21	1.90	2.00	2.75	3.99	4.56	**5.14**	4.65
36TH HOUR,	30	1.41	2.38	2.56	3.19	3.76	3.24	3.11	4.63	5.22	5.93	**6.18**	5.47
INCLUSIVE)													
5TH	10	0.29	0.44	0.45	0.37	0.60	0.61	0.83	1.05	1.06	**1.15**	1.11	1.04
TEST	15	0.50	1.01	1.02	0.87	0.91	1.15	1.34	1.71	2.41	2.90	**3.34**	2.55
INTERVAL	20	0.80	1.41	1.54	1.55	1.43	1.67	1.83	2.58	3.81	4.70	**5.01**	4.86
(37TH TO	25	1.23	2.14	2.30	2.93	2.97	2.45	2.75	3.87	4.94	5.66	**6.30**	5.72
48TH HOUR	30	1.48	2.70	3.08	3.72	4.66	4.00	4.34	5.81	6.14	6.67	**6.87**	6.38
INCLUSIVE)													
NUMBER OF EXPERIMENTS		3	2	2	2	4	7	3	2	4	3	3	3

12 hours (24th to 36th hour); 5th interval, the last 12 hours of the experiment period (37th to 48th hour). Each line of the table shows values for a single temperature and each column shows values for a single oxygen pressure. The number of experiments on which the values of each column are based is shown at the bottom of the column. The probable errors of the averages were computed in a large number of cases by BESSEL'S (30) formula, and they were found in most cases to be less than 4 per cent. of the average itself; in a very few cases the probable error was about 6 per cent. of the average. It is probably safe to suppose that the lowest values given in table II represent the facts within plus or minus 0.02 or 0.03 mg. and that the highest values are thus representative within plus or minus 0.4 or 0.5 mg.

From the averages given in table II, the most obvious facts to be noted are that these experiments gave rates of CO_2 production varying in magnitude from below 0.3 mg. to nearly 7.0 mg. per hour, for 100 seedlings, and that the magnitudes of the rates are clearly related to the temperature-oxygen combination used and to the developmental phase of the plantlets.

PROGRESSIVE INCREASE IN CO_2 PRODUCTION THROUGHOUT THE EXPERIMENT PERIOD.—It is generally evident from table II that the rate of CO_2 production for any combination of temperature and oxygen pressure became progressively greater with time; the rate for a later interval generally more or less exceeds the corresponding rate for any earlier interval in the same experiment. This would be expected, for the rate here considered should generally be greater as development of the plantlets proceeds through its earlier phases. The developmental progress of CO_2 production must have been very slow at the inception of germination and this progress in the experiment period fails to be indicated or was only slightly marked for combinations of low temperature and low oxygen pressure. This progress is generally well shown, however, for combinations of low temperatures and high oxygen pressure and for combinations of high temperatures and any oxygen pressure tested, being most pronounced for the combination of 30° and 90 or 95 per cent. of oxygen in the gas mixture.

The time graphs of figures 3 and 4 are representative samples of a complete series of graphs representing all the data in table II. Figure 3 shows the five time graphs, one for each of the temperatures tested, for each of six representative oxygen pressures; namely, 9.8, 20, 50, 75, 90 and 98.3 per cent. Each section of the figure shows the numerical data for CO_2 production (ordinates) for each of the observation intervals (abscissae), there being five graphs in each case, one for each of the maintained temperatures used. The temperature represented is shown at the right-hand end of each graph. Each of these single graphs thus shows the time march of CO_2 production from the first interval (3rd to 6th hour after the

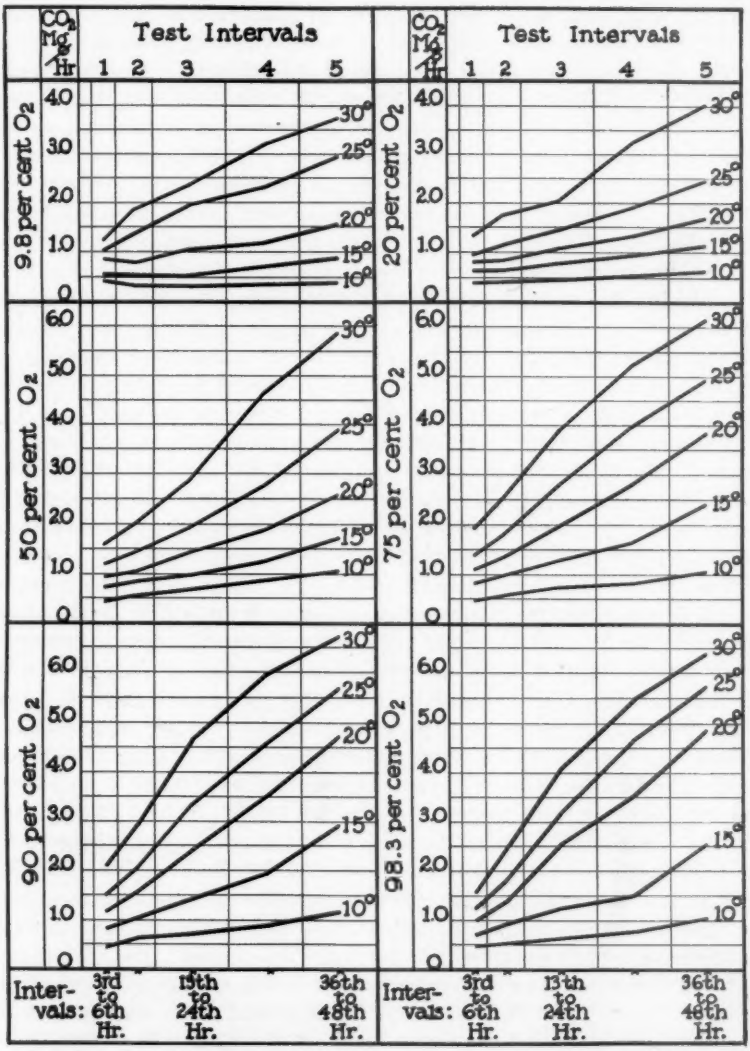

FIG. 3. Representative time graphs showing the march of CO_2 production during the experiment period for each temperature as related to oxygen pressure. These are arranged in groups, each group representing a different oxygen percentage.

start of the experiment) to the fifth interval (37th to 48th hour). In each of the time graphs (including those of figure 4 as well as those of figure 3) the abscissa for any point represents the middle of the observation interval in question; for example, data for the second interval (7th to 12th hour of the period) are plotted as referring to the end of the 9th hour.

In no section of the series of graphs represented by figure 3 is there any crossing or coincidence of the graphs; with every oxygen pressure tested the five-degree temperature intervals were amply great to insure perfectly distinct graphs of this sort and it is apparent that much narrower temperature intervals might have been employed without the introduction of confusion due to unexplained variability among the lots of seedlings. Of course the five graphs for any oxygen pressure approach one another for the shorter abscissae, since the rates of CO_2 production must all have approached zero at the beginning of the germination period, no matter what combination of temperature and oxygen pressure was used.

The graphs of figure 4 are in part the same graphs as are shown in figure 3. In figure 3 there is for each one of six representative *oxygen pressures* a group of five time graphs (*for the five temperatures*) and each section of the figure represents *a single oxygen pressure*. In figure 4, on the other hand, there is for each of the five *temperatures* a group of four or five time graphs (*for representative oxygen presures*) and each section of this figure represents *a single temperature*. By the first arrangement the graphs are grouped according to oxygen presure while they are grouped according to temperature by the second arrangement. In figure 4 both the lowest and the highest graph are shown for each temperature, with one or two intervening graphs for representative oxygen pressures between the pressure for the lowest and that for the highest graph. These are all full lines. For 10° they are for oxygen pressures of 0.6, 20 and 90 per cent. and for the other temperatures they are for 0.6, 20, 50 and 95 per cent. (In the few instances where the same graph is not highest with respect to all intervals the graph shown as highest is the one that is highest for the fifth interval.) In addition, the graph for the oxygen pressure of 98.3 per cent. is shown in every case, its line being broken to distinguish it more readily from the other graphs.

Some temperature-oxygen combinations failed to show a regular increase in the rate of CO_2 production from interval to interval and these exceptions are of special interest. They are listed here for the sake of inspection. Each combination is shown, in the following lists, by its temperature and its oxygen-percentage value separated by a comma, and the value in parenthesis is the difference (in hundredths of a milligram) between the two average rates considered. For the following thirteen combinations of temperature and oxygen pressure the average for the second interval

does not exceed that for the first: 10°, 0.6 (10); 10°, 3.1 (5); 10°, 6.3 (7); 10°, 9.8 (8); 10°, 16 (3); 15°, 0.6 (5); 15°, 3.1 (3); 15°, 6.3 (5); 15°, 9.8 (2); 15°, 16 (2); 20°, 0.6 (2); 20°, 3.1 (0); 20°, 9.8 (6). For the following eight combinations the average for the third interval does not exceed that for the first: 10°, 0.6 (9); 10°, 3.1 (8); 10°, 6.3 (4); 10°, 9.8 (9);

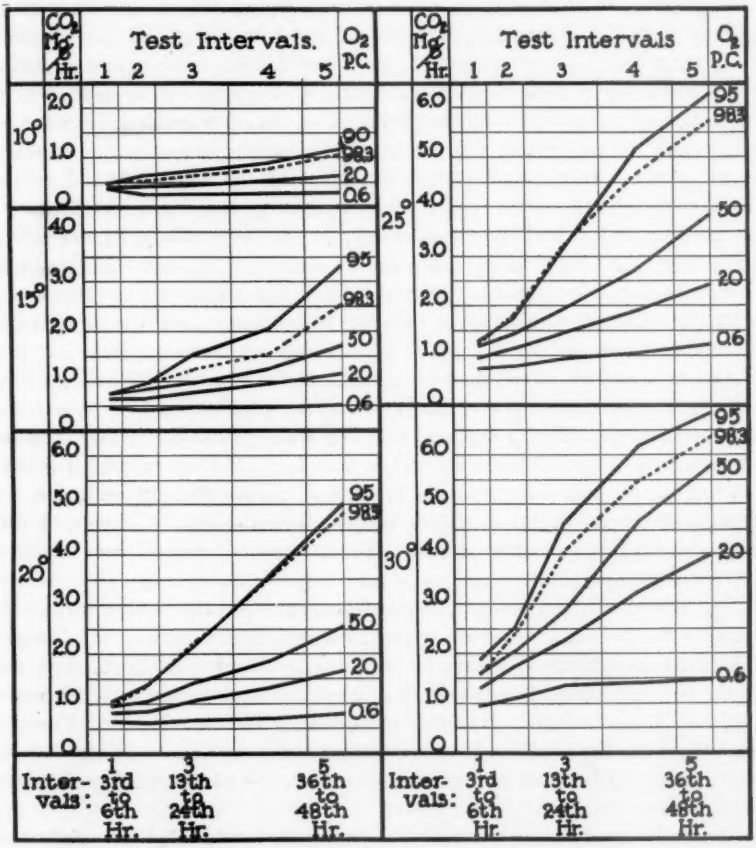

FIG. 4. Representative time graphs showing the march of CO_2 production during the experiment period for each oxygen pressure as related to temperature. These are arranged in groups corresponding to the several temperatures, each group representing a different temperature.

10°, 16 (0); 15°, 0.6 (0); 15°, 6.3 (0); 15°, 9.8 (4). For the following four combinations the rate for the fourth interval does not exceed that for the first: 10°, 0.6 (8); 10°, 3.1 (3); 10°, 6.3 (1); 10°, 9.8 (6). Only for

the combination of 10° and 0.6 per cent. does the rate for the fifth interval fail to exceed that for the first. These exceptions are all confined to temperatures of 20° or lower and to oxygen pressures of 16 per cent. or lower and they correspond to environmental complexes that gave relatively low rates of CO_2 production in any interval. In some of these instances the exceptions may be due to possible errors in observation, which were of course relatively larger for these lowest rates, but many of them appear to be significant, suggesting that the effect of the environmental change from germination flask to culture flask (or the lagging influence of the environmental conditions of the germination period extending into the experimental period) required more or less of the experiment period in which to disappear. It is possible that the effect of the environmental change itself may have been important in determining the higher rate of CO_2 production in the earlier test intervals, in the cases where this occurred. It has been mentioned in various connections in the literature that a sudden change in the environment may result in a stimulation or retardation of activity, either of which may be opposite in tendency from the direct effect of the conditions prevailing after the change. A recent notable example of this is contained in a paper by PARIJA (**34**), in which the graphs illustrate pronounced temporary increases in CO_2 production by apples when they were subjected to a change from one atmospheric environment to another with a lower oxygen percentage, and a temporary reduction in CO_2 production for an environmental change of the opposite description, for changes between several different oxygen percentages, including ordinary air, pure oxygen, pure nitrogen, and percentages of oxygen from 3 to 9 at intervals of 2 per cent.

In this connection we may neglect the data for the first interval and consider the period of transfer and adjustment as of 8 rather than 2 hours. When that is done an examination of table II shows only three exceptions of the sort here considered; only for the three combinations, 10°, 3.1 (3); 10°, 9.8 (1) and 15°, 9.8 (2), were the rates for the second interval greater than those for the third and these exceptions are negligible. Rates for the second interval were in all cases smaller than the corresponding rates for the fourth or fifth.

Because of the progressive increase in the rate of CO_2 production throughout the period, the time graphs generally slope upward from low ordinates at the left to higher ones at the right. The mean rate of upward slope is usually greater with higher temperature than with lower, for any oxygen pressure. It is usually greater with higher oxygen pressure than with lower, for any temperature, but generally there is indicated an optimal oxygen pressure below 98.3 per cent. In no case does a graph bend downward at the right, which indicates that no maximum rate of CO_2 production

was attained in the experiment period with any of these complexes of conditions. In some cases the graphs beyond the second interval are nearly rectilinear (*e.g.*, that for 25° and 20 per cent.) in other cases they are somewhat concave upward in the region of the later intervals, and in still other cases they are somewhat convex upward in that region. Of course upward convexity means a decreasing acceleration of the rate of CO_2 production and suggests that a maximum in that rate may have been approached. This last suggestion applies especially to the graphs for 30°, a temperature that must be regarded as surely supra-optimal for health in such seedlings as these, for it is likely that pathological conditions supervened before the close of the experiment period in all cultures maintained at 30°.

In each section of figure 4 the upward slope of the time graphs is seen to be generally steeper for any temperature as the oxygen pressure is greater, until the highest graph for the given temperature is reached, but the graph for a pressure of 98.3 per cent. (broken line) regularly lies below the highest graph. The highest oxygen pressure (nearly pure oxygen) gave generally lower average rates of CO_2 production for any temperature and observation interval than did the slightly lower pressure that gave the corresponding highest rate for that temperature and interval. In the whole series of time graphs represented by figure 4 the only exceptions to this rule are for 10° in the first interval. The rates for 10° in the first interval are low and alike for 90, 95 and 98.3 per cent. For 25° in the second and third intervals the graph for 98.3 per cent. lies slightly above that for 95 per cent., but in these two instances the highest rates were given by an oxygen pressure of 90 per cent. and reference to table II shows that these two ordinates of the graph for 98.3 are both much smaller than the corresponding values for 90 per cent.

SPECIAL STUDY OF THE COMBINATION OF 20° AND THE OXYGEN PRESSURE OF 20 PER CENT. IN THE SEVERAL INTERVALS.—Special attention may be called to the behavior of the seedlings subjected to the maintained combination of 20° and 20 per cent. The values for this combination of a commonly experienced temperature and ordinary air may be considered as fairly representative of many germination tests and of what occurs when such seed as was used in this study is allowed to germinate in field plantings. These seedlings had been transferred from water to the standard nutrient solution at the beginning of the experiment but their temperature and oxygen pressure had not been significantly different during the experiment period from what these had been during the germination period. For the youngest development phase studied (1st interval, when shoots were only about 0.5 mm. long and roots were barely showing through the coleorhiza) the average mean rate of CO_2 production was 0.82 mg. per hour per 100

seedlings. The rate increased as time went on (being 0.84 mg. for the 2nd interval, 1.07 mg. for the 3rd interval, 1.33 mg. for the 4th interval) until it had a value of 1.67 mg. for the last interval, when the seedlings had shoots about 5 mm. long and roots about 20 mm. long. It is remarkable that this series of values, from 0.84 for the 2nd interval (say 9 hours after the beginning of the experiment), to 1.67 for the 5th interval (say 42 hours after the beginning of the experiment), shows an increase that is almost proportional to time, at the rate of about 0.025 mg. per hour.

RATES IN THE SECOND INTERVAL.—Because some of the rates of CO_2 production in the first interval appear to show influence of the environmental change that occurred when the cultures were started, the earliest interval which is practically without such inconsistencies is the second, the data for which may be taken to represent these seedlings at a very early developmental stage. These data are shown in the second horizontal section of table II and by the graphs of figures 5 and 6.

The chart of figure 5 was planned to show how these very young seedlings (all of the same age with respect to time) exhibited different rates of CO_2

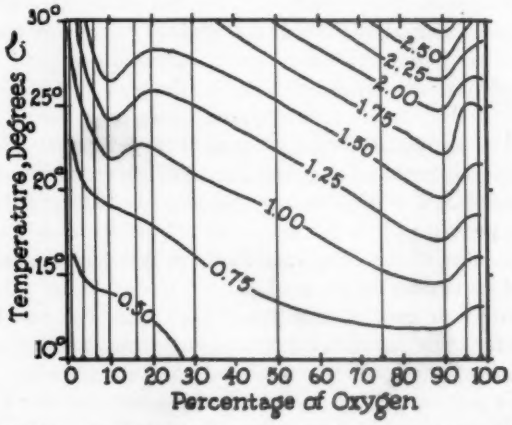

FIG. 5. Diagram showing by contours or isopleths, the relations between rate of CO_2 production (by 100 seedlings) and maintained temperature-oxygen combinations for the second test interval. Points of intersection of the vertical and horizontal lines represent the values given in table II. For all points on any contour the average rate of CO_2 production is the same, its value being indicated by the number on the contour (0.50 to 2.50).

production according to the particular maintained combinations of temperature and oxygen pressure to which they had been subjected since the cultures were started. This chart represents a solid diagram in which "east-west" distances represent progressively larger partial pressures of

oxygen, "south-north" distances represent progressively higher maintained temperatures, and altitudes (as shown by the values on the isopleths or contours) represent hourly rates of CO_2 production. Isopleths are shown for each rate from 0.50 mg. to 2.75 mg., by intervals of 0.25 mg. A glance at this diagram will show what average hourly rate corresponds to any tested combination of temperature and oxygen pressure, or what combinations of these factors correspond to any particular average rate, for the second observation interval. It is notable that the same rate may correspond to many different combinations of temperature and oxygen pressure. For example, the rate of 1.0 mg. per hour per 100 seedlings in the second test interval coresponds to combinations of about: 14.5° and 86 per cent., 17.5° and 58 per cent., 20° and 38 per cent., 22.75° and 18 per cent., 22° and 10 per cent., 28.5° and 0.3 per cent., etc., etc. This manner of presenting the numerical data emphasizes the generalization that neither temperature alone nor oxygen pressure alone is to be considered as the determining condition for any rate of CO_2 production; both of these factors must be considered and there is a broad range of combinations of them that correspond to the same rate.

It is to be noted that the isopleths of figure 5 are in general closer together (*i.e.*, the slope of the surface represented by the diagram is steeper) in the region of combinations of the higher values of the two experimental variables than in the region of combinations of the lower values. In other words, these seedlings were less sensitive, as far as the rate of CO_2 production is concerned, to differences between combinations of high temperature and high oxygen pressure than to differences between combinations of low temperature and low oxygen pressure.

It is interesting to observe also that, for oxygen pressures between about 20 per cent. and about 90 per cent., the temperature corresponding to any average hourly rate of CO_2 production is generally lower as the oxygen pressure is higher, the oxygen pressure corresponding to any rate is lower as the temperature is higher, and the relation between temperature and oxygen pressure for any average rate above about 1 mg. per hour is nearly a linear one and about the same for all these rates. Within the region of the diagram here considered (between oxygen pressure of about 20 per cent. and about 90 per cent.) the percentage value of the oxygen pressure giving any average rate of CO_2 production above about 1 mg. per hour is decreased by about 15 for each temperature increase of about 2°. Rates of CO_2 production below about 1 mg. per hour show contours with progressively lower slopes in the region of approximate linear relation between temperature and oxygen pressure, the left margin of this region being shifted to the right. The characteristic forms of the contours for the region of oxygen pressures below about 20 per cent, and for the region

of pressures above about 90 per cent. bring out other modifications which will be referred to below. Within the range of oxygen pressure between about 20 and about 90 per cent. the rate of CO_2 production for any pressure is shown to increase rather regularly with increase in temperature, somewhat more rapidly in the higher than in the lower temperature ranges. Also, the rate of CO_2 production for any temperature is shown to increase rather regularly with increase in oxygen pressure within the specified range, somewhat more rapidly for higher than for lower temperatures.

Other diagrams similar to that of figure 5, for the data of the 3rd, 4th and 5th intervals and for the experiment period as a whole are just as interesting and instructive as the one here shown. They may be readily constructed from the data given in table II.

Figure 6 presents plane graphs of the data on CO_2 production in the second interval. Ordinates represent average hourly rates of CO_2 production and abscissae represent percentages of oxygen in the gas mixture.

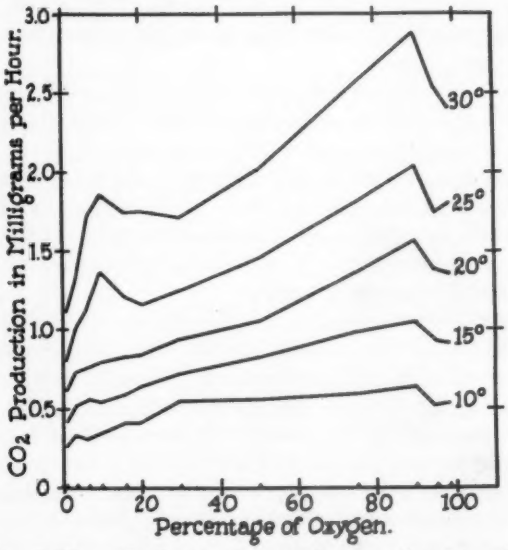

FIG. 6. Graphs of CO_2 production in the second observation interval, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

There are five graphs in each group, one for each temperature tested.

Two graph maxima are clearly shown for 25° and 30° and suggested for 10°, 15° and 20°. For 25° and 30° the first graph maximum corresponds to a pressure of 9.8 and in all cases the second graph maximum

corresponds to a pressure of 90 per cent. Between these two graph maxima there is indicated a minimum region, the minimum itself corresponding to 20 per cent. for 25° and to 16–30 per cent. for 30°.

The existence of two graph maxima implies two optimal oxygen pressures for the given temperature, the first (lower) optimal pressure being 9.8 per cent. for 25 and 30° and the second (higher) optimal pressure being 90 per cent. The second or main optimum of pressure is clearly shown for all temperatures tested in the second interval, but the first optimum is only suggested for the three lower temperatures, for which it appears to be progressively lower with lower temperature. The double optimum of oxygen pressure is also indicated by the contours of figure 5; the graphs of figure 6 are of course simply "east-west" profiles of the area represented by figure 5. More attention will be given to this double optimum in connection with the discussion of the rates for the entire period, in the next following section.

Another aspect of the complex set of relations shown by figures 5 and 6, for the second observation interval, may be illustrated by noting that although the rate of CO_2 production is shown to be generally higher with higher temperature and also with higher oxygen percentage, within the ranges studied, yet the two influences are not generally equally effective. The temperature influence is most effective with an oxygen pressure of about 90 per cent. and least effective with a pressure of 0.6 per cent., but it is more effective with 10 than with 20 per cent. and less effective with 98.3 than with 90 per cent. The plantlets were able to produce CO_2 most rapidly, in this second interval, when the temperature effectiveness was high; that is, when a given increase in temperature gave a large increase in CO_2 production.

The rate of CO_2 production for any temperature in the second interval is shown by the plane graphs to have been nearly proportional to the oxygen pressure for a range of pressure between about 20 or 30 per cent. and about 90 per cent., the upward slope of the nearly rectilinear graphs for that range being progressively steeper with higher temperature. A similar observation has been noted from the isopleths of figure 5.

CO_2 PRODUCTION IN THE ENTIRE EXPERIMENT PERIOD.—The data for total CO_2 production in the whole experiment period are given in table III and are represented by the graphs of the first part of figure 7. The arrangement of these graphs is like that of the graphs in figure 6, ordinates being the average rates of CO_2 production while abscissae are oxygen pressures, with a separate graph for each of the five temperatures. The units shown at the left of the figure are for average hourly rates for the whole period; *i.e.*, each total value given in table III has been divided by 46 (the number of hours in the experiment period) to give the corresponding ordinate shown in the graphs.

TABLE III

TOTAL CO_2 PRODUCTION BY 100 SEEDLINGS FOR EACH COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN THE ENTIRE EXPERIMENT PERIOD

TEMPERATURE	AMOUNT OF CO_2 PRODUCED IN 46 HOURS (BY 100 SEEDLINGS) WITH OXYGEN PRESSURE OF											
	0.6 PER CENT.	3.1 PER CENT.	6.3 PER CENT.	9.8 PER CENT.	16.0 PER CENT.	20.0 PER CENT.	30.0 PER CENT.	50.0 PER CENT.	75.0 PER CENT.	90.0 PER CENT.	95.0 PER CENT.	98.3 PER CENT.
Deg. C.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
10	12.8	16.6	17.0	16.6	22.5	23.0	30.3	36.3	36.8	**39.1**	35.4	34.5
15	21.6	37.2	35.4	30.3	33.1	40.8	45.5	55.2	73.6	82.3	**91.5**	72.2
20	31.3	51.9	55.7	52.4	51.9	57.1	63.0	80.4	115.8	141.2	**142.1**	139.8
25	46.0	75.4	84.6	98.4	93.8	80.4	88.3	115.8	157.3	180.2	**190.8**	178.3
30	61.6	96.6	110.3	127.3	144.3	130.2	131.4	178.3	205.1	**232.6**	228.6	211.4

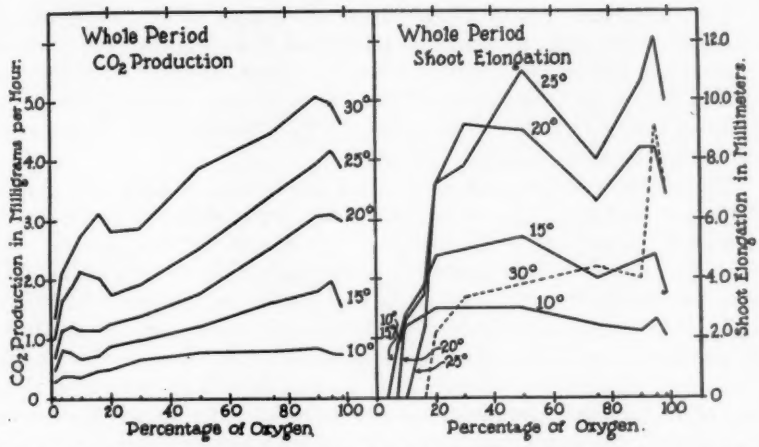

FIG. 7. Graphs of CO_2 production and of shoot elongation in the entire experiment period, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

The graphs for the entire period are very similar to those for the second interval. The two graph maxima shown for the second interval are clearly shown here for 15°, 20°, 25° and 30° and are suggested for 10°. The portion of each graph between the subordinate minimum and the second maximum is nearly rectilinear, with the slope progressively steeper as the temperature is higher, as is true of the graphs for the higher temperatures in the second interval. The first graph maximum corresponds to higher oxygen pressure with higher temperatures; for 10° and 15° it is indicated as corresponding to 3.1 per cent., for 20° it corresponds to 9.8 per cent., and for 30° it corresponds to 16 per cent. These pressure values are those of the first or subordinate optimal pressure. The second graph maximum corresponds to a pressure of 90 or 95 per cent. for all temperatures and this slight variability appears not to be related to temperature. The abscissa of the second graph maximum is of course the main or second optimal oxygen pressure in each instance.

It is specially remarkable that the main optimal oxygen pressure is shown as below 98.3 per cent. for every temperature tested in every interval and in the whole period; this highest pressure tested was surely supra-optimal in every instance. Reference to table II shows that the main optimal oxygen pressure (the average rates for which are there shown in bold-face type) was either 90 or 95 per cent. in every case excepting 10° in the first and third intervals, for which it is shown as 75 per cent.

The subordinate graph minimum for the graphs of the whole period generally corresponds, like the first maximum, to an oxygen pressure that is somewhat higher as the temperature is higher. For 10° and 15° its abscissa is 9.8 per cent., for 20° the corresponding oxygen pressure is shown as between 9.8 and 16 per cent., for 25° its abscissa is 20 per cent. and for 30° between 20 and 30 per cent.

The existence of two maxima with an intervening minimum in graphs of the sort shown in figure 6 and the first part of figure 7 seems not to have been pointed out in the literature before and the now obscure temperature-oxygen relations that must be responsible for these peculiar features are worthy of special study. An explanation is doubtless to be sought in the physiological chemistry of respiration in such seedlings as these, but no complete and plausible hypothesis seems as yet to be possible in this connection. An apparently pertinent suggestion will be presented after the graphs for shoot elongation have been considered. As has been mentioned, these two graph maxima imply a double optimal oxygen pressure for each temperature, the first (or lower) optimal pressure being in each instance the abscissa for the first graph maximum, while the second (or higher, main) optimal pressure is the abscissa for the second graph maximum. The physiological meaning of this apparently important double optimal pressure may be made clear by a concrete example, as follows: For the whole experiment period and the temperature of 25° the oxygen pressure of 9.8 per cent. gave a much higher rate of CO_2 production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. are shown as about alike. The rate of CO_2 production for 25° and 6.3 per cent. of oxygen was 84.6 mg. and that for 25° and 20 per cent. of oxygen was 80.4 mg., while that for 25° and 9.8 per cent. of oxygen was 98.4 mg.

Graphs are shown here for only the second interval and the entire period, but the data of tables II and III show that the double graph maximum and the intervening minimum occur rather regularly for other intervals than the second, though the data are not wholly consistent in this respect. In table IV are assembled the approximate oxygen-pressure values corresponding to the first maximum, the second minimum and the main maximum in each graph, including the graphs not here reproduced as well as those of figure 6 and the first part of figure 7. The data are from tables II and III.

With two exceptions (30°, first interval, and 20°, second interval) the double graph maximum, which implies a double optimum in oxygen pressure, is at least suggested for all temperatures tested in all observation intervals and in the whole period and it is clearly shown for the higher temperatures in every case. Both the first optimal pressure (correspond-

TABLE IV

APPROXIMATE OXYGEN PRESSURES (PERCENTAGE OF OXYGEN IN THE GAS STREAM) CORRESPONDING TO FIRST OR SUBORDINATE MAXIMUM, SECOND OR SUBORDINATE MINIMUM AND SECOND OR MAIN MAXIMUM ON EACH GRAPH OF CO_2 PRODUCTION FOR EACH TEMPERATURE TESTED IN EACH OBSERVATION INTERVAL AND IN THE ENTIRE EXPERIMENT PERIOD. THE GRAPHS FOR THE SECOND INTERVAL AND THE ENTIRE PERIOD ARE REPRODUCED IN FIGURES 6 AND 7

		10°	15°	20°	25°	30°
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*
1st interval	1st max.	16	6.3	9.8	9.8	.....
	2nd min.	20	9.8	16	20	.....
	2nd max.	75	90	90	90	90
2nd interval	1st max.	3.1?	6.3?	.....	9.8	9.8
	2nd min.	6.3?	9.8?	.....	20	30
	2nd max.	90	90	90	90	90
3rd interval	1st max.	6.3?	6.3	6.3	9.8	16
	2nd min.	9.8?	9.8	16	20	30
	2nd max.	75	95	90	90	90
4th interval	1st max.	6.3?	6.3	6.3	9.8	16
	2nd min.	9.8?	9.8	16	20	30
	2nd max.	90	95	95	95	95
5th interval	1st max.	6.3	6.3	9.8	16	16
	2nd min.	9.8	9.8	16	20	20
	2nd max.	90	95	95	95	95
Whole period	1st max.	3.1?	3.1	6.3	9.8	16
	2nd min.	9.8?	9.8	9.8—16	20	20—30
	2nd max.	90	95	90—95	95	90

ing to the first graph maximum) and the pressure corresponding to the second graph minimum are generally higher with higher temperature and with later intervals. Of course the pressure corresponding to the second minimum on a graph is always somewhat higher than that corresponding to the first maximum, the latter pressure being the one here called the first optimal pressure. Although some inconsistencies are to be noted, it seems clear from table IV that the double optimum of oxygen pressure for CO_2 production is significant and that the indications in this respect that are shown by the graphs of figures 6 and 7 are truly representative of the entire series of graphs.

The five graphs of CO_2 production in the whole period are also like those for the same process in the second interval in that each one has a

nearly rectilinear region lying between the second or subordinate minimum and the second or main maximum; for oxygen pressures between about 20 or 30 per cent. and about 90 per cent., CO_2 production was nearly proportional to oxygen pressure and the constant of proportionality (the upward slope of the graphs) is progressively greater with higher temperature, its value being apparently determined by a temperature and the current development phase of the plantlets.

Shoot elongation in the entire experiment period

Observations on the rates of enlargement exhibited by the seedlings of the various cultures were not sufficiently precise for use in studying the several observation intervals of the experiment period but the average final length of the longest ten shoots of each culture furnishes a numerical value that may represent the corresponding total shoot elongation for the entire period. These average final lengths are given in table V. Where no value is shown, elongation was too slight to be measured by the rather crude method employed. All values in any line of the table are for the same oxygen pressure and the highest value for each pressure is designated by the use of bold-face type. An asterisk is shown at the left of the highest growth value for each temperature (highest value in each column) and a vertical line at the right brackets several values when these appear to indicate an optimal range of oxygen pressure for the temperature in question.

Besides the graphs of CO_2 production as related to temperature and oxygen pressure in the entire experiment period, figure 7 presents also the corresponding graphs for shoot elongation, the data for the latter being taken from table V. Ordinates of these growth graphs are indices of total shoot elongation for the entire experiment period and abscissae represent percentages of oxygen in the gas mixture. There is a graph for each temperature tested.

The graphs of shoot elongation differ from the corresponding ones of CO_2 production in several ways. While no main minimal oxygen pressure is shown for CO_2 production with any temperature, all five of the graphs of shoot elongation are seen to meet the axis of abscissae at the left. It is important to note that the highest oxygen pressure giving no measurable growth (a pressure slightly below the minimal pressure for shoot elongation) is progressively greater with higher temperatures. For 10° and 15° this is 3.1 per cent., for 20° it is 6.3 per cent., for 25° it is 9.8 per cent. and for 30° it is 16 per cent.

Each of these graphs of shoot elongation shows three low or minimal regions and two high or maximal regions on the oxygen scale, as in the case of the corresponding graphs of CO_2 production, but details are very

TABLE V

AVERAGE LENGTHS OF TEN LONGEST SHOOTS IN EACH CULTURE, AT END OF EXPERIMENT, AS RELATED TO TEMPERATURE AND PARTIAL PRESSURE OF OXYGEN

PARTIAL PRESSURE OF OXYGEN	AVERAGE FINAL LENGTH OF TEN LONGEST SHOOTS IN EACH CULTURE, FOR TEMPERATURE OF				
	10° C.	15° C.	20° C.	25° C.	30° C.
per cent.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
0.6	.....	.....	.....	.....	.....
3.1	.....	.....	.....	.....	.....
6.3	1.6	1.6	.....	.....	.....
9.8	2.4	2.8	2.6	.....	.....
16.0	2.8	3.8	3.0	2.4	.....
20.0	3.0	4.8	7.2	7.2	2.2
30.0	3.0	5.0	*9.2	7.8	3.4
50.0	3.0	*5.4	9.0	11.0	3.8
75.0	2.4	4.0	6.6	8.0	4.4
90.0	2.2	4.6	8.4	10.6	4.0
95.0	2.6	4.8	8.4	*12.2	*9.2
98.3	2.0	3.6	6.8	10.0	6.8

* Highest growth value for each temperature.

different for the two processes. Each growth graph slopes sharply upward at the beginning, quickly attaining the first or subordinate graph maximum. Each graph then slopes downward to the second or subordinate graph minimum and then again upward to the second or main graph maximum, beyond which it slopes sharply downward to its end.

The presence of two graph maxima with an intervening subordinate minimum renders these graphs of shoot elongation peculiarly interesting and makes it necessary to consider (as in the case of the corresponding graphs of CO_2 production) two optimal oxygen pressures for each temperature. The remarkable similarity in form of all the growth graphs makes it seem probable that this feature is highly significant and important. (It is to be borne in mind, however, that the data of shoot elongation were secured by a much less precise technique than that by which the data of CO_2 production were secured.) These graphs all agree in showing the second or main maximum with an oxygen pressure of 95 per cent. (90–95 per cent. for

20°). The pressure of 98.3 per cent. was clearly supra-optimal for shoot elongation, as well as for CO_2 production, with every temperature. The first graph maximum for shoot elongation corresponds to a pressure range of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°; to a pressure of 30 per cent. (or perhaps a pressure range of 30–50 per cent.) for 20°; and for 30° this maximum is suggested for a pressure of 75 per cent. The intervening second minimum is remarkably well and consistently shown by the graphs for 15°, 20° and 25°, on which it corresponds to a pressure of 75 per cent. It is indicated as corresponding to a pressure of 90 per cent. on the graphs for 10° and 30°, however, temperatures that are respectively below and above the temperature most favorable to growth. The 30-degree graph remains exceptionally low for all percentages of oxygen until the abscissa for 90 per cent. is passed, when it ascends sharply to its maximum, for 95 per cent.

As in the case of CO_2 production, no reference to a double optimum of oxygen pressure for growth seems to have appeared in the literature and this feature of growth control in seedlings such as were used in this study is worthy of special attention, notably in connection with the corresponding double optimum of oxygen pressure for CO_2 production and all that is implied by such occurrences.

Some comparisons between CO_2 production and shoot elongation as these were related to temperature and oxygen pressure

Possible competition between CO_2 production and shoot elongation.—In figure 7 it is seen that the pressure corresponding to the first or subordinate maximum on the graphs of CO_2 production for the whole experiment period is apparently the same as the highest oxygen pressure for which the corresponding growth graph has zero as ordinate. For the several temperatures these critical pressures are as follows: 10° and 15°, 3.1 per cent.; 20°, 6.3 per cent.; 25°, 9.8 per cent.; 30°, 16 per cent. CO_2 production was regularly more rapid with higher oxygen pressures up to the pressure that just allowed measurable shoot elongation, beyond which CO_2 production was notably lower with still higher pressures until the subordinate minimal region of the CO_2 graphs is passed. Although a considerable rate of CO_2 production may be supposed to be necessary for growth in such seedlings as these, yet the occurrence of growth may retard CO_2 production, as might be true if the two processes were competing, as it were, for the same plastic materials. The possibility that such competition may exist between growth and respiration were suggested by Copeland (**9**) who said that "the more active the respiration, the less the relative amount of plastic material left available for growth." That the minimal oxygen pressure required for growth is greater with progres-

sively higher temperatures within the range considered, is in agreement with the results of CANNON's (7) experiments on the oxygen requirement of roots, and it appears that the higher temperatures may promote respiration more than they promote growth, other conditions being suitable. Of course the rate of CO_2 production should tend to increase with the growth rate when the latter is relatively great, for respiration must be supposed to proceed more rapidly as the number of active cells (or the volume of active protoplasm) increases with development and this may perhaps explain the second maxima in these curves. The highest percentage of oxygen tested generally gave somewhat lower rates of both shoot elongation and CO_2 production than did a slightly lower percentage with the same temperature. But neither growth nor CO_2 production approached zero rate with any tested temperature combined with the highest oxygen pressure tested, 98.3 per cent.

From the graphs in figure 7 it is apparent that the efficiency of respiration with respect to the amount of growth produced became greater as the oxygen pressure was increased above the main oxygen pressure minimum for growth, until the subordinate minimum of oxygen pressure for CO_2 production was passed. Above this region in the graph the efficiency of respiration gradually decreased until the subordinate minimum of oxygen pressure for growth was reached. For temperatures near the optimum for growth, the maximum efficiency of respiration was reached at oxygen pressures near that in ordinary air.

KOSTYTSCHEW points out (**25,** p. 54) that CO_2 production may not be greatly reduced by low partial pressures of oxygen in the surroundings, though the energy yield from respiration under such circumstances must be much less than when the oxygen pressure is higher. A rise in temperature may increase the respiration rate to a greater degree than it increases either the growth rate or the rate of transformation of plastic substances to available forms. The rate of growth may thus be limited on the one hand by the supply of available energy and on the other hand by the supply of plastic substances not used up in the respiration process.

TEMPERATURE INFLUENCE COMPARED WITH OXYGEN INFLUENCE WITH RESPECT TO CO_2 PRODUCTION AND SHOOT ELONGATION, BY MEANS OF COEFFICIENTS OF EFFECTIVENESS.—An idea of the relative influences of temperature and oxygen pressure on the rates of CO_2 production and shoot elongation, as shown by the results of these experiments, may be obtained by selecting an environmental complex that gave low rates and then ascertaining how much greater the rates were when one or the other or both of these two experimental variables were altered so as to give the greatest effect. What may be called coefficients of effectiveness (LIVINGSTON and SHREVE (**29,** p. 208) may be secured in this way for alterations of one or

more environmental conditions. We may take as our unit for shoot elongation the value 3.0 mm., which corresponds, let us say, to the temperature-oxygen combination of 10° and 20 per cent. (table V). With the tem-temperature increased from 10° to 25° (the oxygen concentration being unchanged) the elongation rate was 7.2 mm., which is about 2.4 times our unit. On the other hand, when the temperature was not altered but the oxygen pressure was increased from 20 per cent. to 95 per cent. the rate value was only 2.6, which is lower than our unit, and the effectiveness of this oxygen-pressure change is consequently negative, its coefficient being 0.9. When both changes occurred together the rate was actually changed from 3.0 to 12.2 mm. and the coefficient of effectiveness of the double change is 4.7, which is 2.14 times as great as the calculated value, 2.2 (2.4 x 0.9). If the two environmental changes applied together had operated just as they did when applied separately, without mutual influence of antagonism or synergism (*i.e.*, if the effects had been simply additive), then the rate corresponding to the double change should have been 2.2 times as great as our unit. That the data actually show the coefficient of effectiveness of the double change as 4.7 instead of 2.2 indicates that when both changes were applied together the influence of one or both was greater than when they were applied separately. The two influences were synergistic in their action and the synergism resulted in an increase of effectiveness from 2.2 to 4.7.

For CO_2 production (table III) these same changes give 3.5 and 1.5 as the coefficients of effectiveness of the temperature alteration and of the oxygen-pressure alteration, respectively, while the coefficient of the double change is 8.3, which is 1.57 times the product of 3.5 and 1.5. These considerations show that the change from the temperature-oxygen combination of 10° and 20 per cent. to the combination of 25° and 95 per cent. was relatively much more effective to give more rapid CO_2 production than it was to give more rapid shoot elongation. Also it is indicated that, for both processes, the temperature change was much more important in this double alteration than was the oxygen change and that there was a pronounced synergistic action on both shoot elongation and CO_2 production, this being nearly twice as great for CO_2 production as for shoot elongation.

Such considerations as these may be valuable for comparisons when further experimental data become available on the effectiveness of temperature and oxygen pressure in controlling the rates of CO_2 production and of growth in organisms. For the present we may simply call attention to the possibilities of this method of analysis and give emphasis to the conclusions just presented.

THE CARDINAL VALUES OF TEMPERATURE AND OXYGEN PRESSURE FOR CO_2 PRODUCTION AND SHOOT ELONGATION IN THE ENTIRE EXPERIMENT PERIOD

FOUR SETS OF VALUES CONSIDERED.—The relations of temperature and oxygen pressure to CO_2 production and shoot elongation may be studied by comparing the two sets of cardinal values for the two experimental variables. The values to be considered are (1) the main minimal, optimal and maximal temperature (A) for CO_2 production and (B) for shoot elongation, and (2) the same critical value of oxygen pressure. From the data of tables III and V (figure 7) the three cardinal values of temperature for each oxygen pressure and the three cardinal values of each oxygen pressure for each temperature are first to be ascertained as nearly as possible.

CARDINAL VALUES FOR CO_2 PRODUCTION.—The minimal temperature for CO_2 production in the entire period was evidently below 10° for every oxygen pressure tested, presumably for every oxygen pressure below that of pure oxygen under 1 atmosphere of gas pressure. The maximal temperature for CO_2 production in the whole period was evidently above 30° for all oxygen pressures, but 30° is surely somewhat supra-optimal for health in seedlings like these and it is probably safe to suppose that that temperature was about the maximum for CO_2 production *in health*. With the behavior of the seedlings under injurious or lethal conditions we are not here concerned.

The optimal temperature for CO_2 production in the whole period was 30° or above for all oxygen pressures tested, but because 30° is to be considered as generally supra-optimal for health we may say that the optimal temperature for CO_2 production *in healthy seedlings* was, like the corresponding maximal temperature, probably not far from 30° for all oxygen pressures below 100 per cent.

No main minimal oxygen pressure for CO_2 production in the whole period is shown for any temperature. It is clearly shown to have been at least below 0.6 per cent. in all cases, but it seems likely that there is no such thing as a minimal oxygen pressure for CO_2 production in such organisms as these, for CO_2 would probably be produced at a measureable rate in the complete absence of oxygen, at least with the higher temperatures tested.

No maximal oxygen pressure for CO_2 production in the whole period is shown and it is evident that we must consider this critical value as above 100 per cent. for all temperatures tested. It would of course be impossible to have an oxygen pressure above 100 per cent. without at the same time introducing a presumably serious alteration in the environmental background; namely, a total gas pressure of more than 1 atmosphere.

The highest rate of CO_2 production for each temperature is shown in black-face type in table III. Of course the oxygen pressure corresponding to the highest rate for any given temperature is the main optimal pressure for that temperature. This main optimum varied with temperature, being shown as 90 per cent. for 10° and 30° and as 95 per cent. for 15°, 20° and 25°. It is remarkable that this value is indicated as below that of pure oxygen for every temperature tested.

Special attention has already been given to the occurrence of a subordinate optimal and a subordinate minimal oxygen pressure for CO_2 production in the whole period, both with low values, which range from 6.3 and 9.8 per cent. (10° and 15°) to 16 and 20 per cent. (30°).

CARDINAL VALUES FOR SHOOT ELONGATION.—The minimal temperature for measurable shoot elongation in the entire period is seen (table V) to have been somewhat below 10° for every oxygen pressure for which data are available. This agrees with the corresponding observation for CO_2 production. But it is suggested that this minimal temperature for shoot elongation was probably lower for median pressures (20–50 per cent.) than for high or low pressures.

The maximal temperatures for measurable shoot elongation in the whole period is shown as low for low oxygen pressures and higher for higher oxygen pressures, being indicated between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 9.8 per cent., between 25° and 30° for a pressure of 16 per cent., somewhat above 30° for pressure from 20 to 98.3 per cent. and farthest above 30° for a pressure of 95 per cent. These relations are very different from those for CO_2 production, for which no definite maximal temperature is shown for any oxygen pressure, although the temperature maximum was actually above 30° in every case.

The optimal temperature for measurable shoot elongation in the whole period is, like the corresponding maximal temperature, low for low oxygen percentages and higher for higher percentages. This optimum is indicated between 10° and 15° for a pressure of 6.3 per cent., about 15° for pressures of 9.8 and 16 per cent., about 20° for pressures of 20 and 30 per cent., and about 25° for pressures of 50–98.3 per cent. The temperature of 30° was unquestionably markedly supra-optimal for shoot elongation in the entire period, for every oxygen pressure employed. In this respect also the temperature relations of growths were very different from those of CO_2 production, for which no definite optimal temperature is shown for any oxygen pressure, although it was surely above 30° in every case.

The main minimal oxygen pressure for shoot elongation in the whole period varied with temperature, being higher for higher temperatures than for lower ones. It is indicated between 3.1 and 6.3 per cent. for 10° and

15°, between 6.3 and 9.8 per cent. for 20°, between 9.8 and 16 per cent. for 25°, and between 12 and 20 per cent. for 30°. This statement differs greatly from the corresponding statement concerning CO_2 production, for which no minimal oxygen pressure is shown for any temperature.

No maximal oxygen pressure for shoot elongation in the whole period is shown for any temperature and that maximum must have been above 98.3 per cent., if such a maximum existed. This is in agreement with the corresponding statement concerning CO_2 production.

The main oxygen-pressure optimum for shoot elongation in the whole period is shown at about 30 per cent. for 10°, probably between 30 and 50 per cent. for 15° and 20°, between 50 and 95 per cent. for 25° and about 95 per cent. for 30°. The oxygen pressure of 98.3 per cent. was clearly supra-optimal with respect to shoot elongation for every temperature tested. For CO_2 production this main optimal oxygen pressure is shown as 90 per cent. with 10° and 30°, and about 95 per cent. with temperatures from 15° to 25°.

The occurrence of two optimal oxygen pressures (or double optimum) for shoot elongation in the whole period has been considered above, where it is pointed out that CO_2 production and shoot elongation were apparently specifically related, in that the oxygen-pressure for shoot elongation is shown as having for each temperature the same value as the corresponding first optimal pressure for CO_2 production.

Optimal combinations of temperature and oxygen pressure for CO_2 production and shoot elongation.—The optimal temperature-oxygen combination for CO_2 production in the entire period, is shown as the combination of 30° and the pressure of 90 per cent., while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and the pressure of 95 per cent. This constitutes an additional point of difference between the temperature-oxygen relations of the two processes. While the combination best suited to shoot elongation (25° and 95 per cent.) gave 12.2 mm. as the highest index of shoot elongation, the average mean hourly rate of CO_2 production corresponding to this index of elongation is only 190.8 mg., but the optimal combination for CO_2 production (30° and 90 per cent.) gave a corresponding CO_2 production of 232.6 mg. and the total amount of shoot elongation for that same combination of temperature and oxygen pressure is only 4.0 mm. per seedling for the experiment period. If the indices of shoot elongation (12.2 mm.) and CO_2 production (190.8 mg.) are both taken as unity for the combination of 25° and 95 per cent. the relative values of these indices for the combination of 30° and 90 per cent. become 0.3 (shoot elongation) and 1.2 (CO_2 production).

It is interesting to remark that the highest rate of shoot elongation shown may perhaps represent the developmental capacity of these seedlings,

for the background conditions and the duration factor employed. They had the capacity to develop shoots about 12.2 mm. long by the end of the experiment period if they were subjected to the temperature-oxygen combination of 25° and the oxygen pressure of 95 per cent. throughout the period, this development being accompanied by the production of about 1.9 mg. of CO_2 per seedling. None of the environmental complexes tested allowed any higher rate of shoot development but higher rates of CO_2 production actually occurred with combinations of 30° and an oxygen pressure of from 75 to 98.3 per cent. The capacity of these seedlings with respect to CO_2 production in the given period, and without regard to pathological or lethal phenomena probably related to the supra-optimal temperature of 30°, is shown as 232.6 mg. for 100 seedlings (or about 2.3 mg. per seedling), for the combination of 30° and the oxygen pressure of 90 per cent.

These observations constitute rather definite physiological characterizations of the seedlings used in this study and they may be valuable for purposes of comparison when systematic data of the sort presented in this paper shall have become available for other plant forms. We may suppose that any seed or seedling possesses a definite development or growth capacity, or a definite capacity for the production of CO_2, etc., and that an optimal environmental complex will permit the organism to attain its capacity, while failure to attain capacity in the specified time period implies a sub-optimal complex of environmental conditions. Of course it is logically possible that the optimal complex of conditions may be a broad range of complexes, or that there may be several different complexes each of which might allow or promote capacity performance. For performance considerably below capacity many different complexes may be suitable, as has been illustrated by the isopleths for CO_2 production in the second interval (figure 5), but as performance approaches the capacity of the organism the range of suitable complexes should become narrower. An interesting diagram of isopleths for shoot elongation may be constructed from the data of table V, but lack of sufficient space and the rather low degree of precision that characterizes these data have led to the omission of the growth diagram here.

It is possible that these seedlings might have exhibited a still greater capacity to grow or to produce CO_2 if the complex of environmental background conditions had been different; for example, if some toxic or stimulating gas were present in the gas stream. The capacity rate here emphasized is to be taken as holding, as far as present knowledge goes, only for the background complex used in these tests.

The cardinal values of temperature and oxygen pressure for CO_2 production in the several observation intervals

In table II the highest rate of CO_2 production for each temperature is shown in bold-face type and of course these values correspond to the main optimal oxygen pressure for the respective temperatures. A study of these optimal pressures, in connection with the other cardinal values of oxygen pressure and the cardinal values of temperature, as they are alike or different for the several consecutive observation intervals, brings out a number of interesting relations, some of which will be mentioned below.

No minimal or maximal temperature with respect to CO_2 production is shown with any oxygen pressure tested (0.6 to 98.3 per cent.) in any of the five intervals. For all intervals, as for the whole period, the minimal temperature is shown as at least below 10°, and the maximal temperature is shown as above 30°, for every oxygen pressure tested. No optimal temperature is shown for any interval, but this value may be taken in every case as not far from 30° for all oxygen pressures, if only healthy seedlings are considered. Otherwise it may be considerably above 30°.

As in the case of the entire period, no main minimal oxygen pressure is shown for any interval or temperature; but this value was surely below 0.6 per cent. in every case. (As has been noted, there is reason for supposing that there is no such thing as a minimal oxygen pressure for CO_2 production in seedlings such as those here used.) No main maximum oxygen pressure is shown for any temperature in any interval, but this value was clearly above 98.3 per cent. in every case. The main optimal oxygen pressure is shown as varying with temperature and to some extent with the developmental phase of the plantlets. Its value is 90 per cent. for the first three intervals and 95 per cent. for the last two, for all temperatures tested, excepting that it is 75 per cent. for 10° in the second and 3rd intervals, 90 per cent. for 10° in the fourth and fifth intervals, 95 per cent. for 15° in the third interval.

In general, the later intervals are well represented by the second, for which the data have been presented rather fully by isopleths and plane graphs. The occurrence of a double optimum of oxygen pressure for CO_2 production is generally indicated for all temperatures in all intervals, the exceptions being of inconsistent occurrence or confined to temperatures that gave very low rates of CO_2 production with all oxygen pressures.

Summary and conclusion

Nature of the experimentation.—This paper presents the results of an experimental study on CO_2 production and shoot elongation in very young wheat seedlings subjected to 60 different maintained environmental

complexes representing a wide range of temperatures and of partial pressures of oxygen in the culture solution in which the seedlings were submerged. The seedlings used were all very nearly alike, having been produced from selected seeds by means of a standard treatment. Their stage of development is indicated partly by the statement that the coleoptile was protruding less than a millimeter but that no roots had yet appeared, and partly by the specifications of the standard germination procedure that was regularly followed. One hundred seedlings were regularly used in each experiment.

The environmental background, including all the influential external conditions excepting the experimental variables, was very nearly the same for all experiments. This background portion of the maintained environment is described in detail, the main features being that the hundred seedlings of an experiment were submerged in 250 cc. of a standard, weak nutrient solution continually stirred by the passage of gas bubbles during the experiment period and that light was excluded from the culture chambers. The culture solution was not renewed and it doubtless changed in various ways (dependent on the activities of the seedlings) throughout the experiment period, which regularly extended for 46 hours after the close of a 2-hour period of transfer and adjustment, the previous germination period having lasted for 42 hours after the placing of the dry seeds in the water of the germination flask. The experiment period was subdivided into five consecutive observation intervals (of 4, 6, 12, 12 and 12 hours), for each of which the average hourly rate of CO_2 production was ascertained; there are thus available from each experiment five measurements of the rate of CO_2 production, representing different stages of the development of the seedlings, which of course changed internally as development advanced.

The experimental variables were maintained with very little fluctuation throughout the period of experiment, but they differed from experiment to experiment in a definite manner. They were the conditions of temperature and oxygen pressure, in 60 different combinations. Twelve different oxygen pressures were tested with each of five different maintained temperatures: 10°, 15°, 20°, 25°, 30° C. Any oxygen pressure was maintained by allowing a previously prepared mixture of oxygen and nitrogen to bubble continuously (at the rate of 20 cc. a minute) through the 250 cc. of nutrient solution in the culture flask. The twelve different oxygen-nitrogen mixtures contained 0.6, 3.1, 6.3, 9.8, 16, 20, 30, 50, 75, 90, 95 or 98.3 per cent. of oxygen by volume. Commercial gases were used in the preparation of the gas mixtures. The total gas pressure in the space above the solution in the culture flask was very little above the pressure of the outside atmosphere, the excess amounting to not more than 2 or 3 cm. of a mercury column.

Five experiments, all with the same partial pressure of oxygen but differing as to temperature, were carried out simultaneously in each experiment series and each series was repeated at least once before the final average rates of CO_2 production were computed. Some special repetitions near the end of the study showed clearly that the stock of seed used did not deteriorate appreciably while the work was in progress.

The final averages represent the rates of CO_2 production for the respective intervals, expressed as milligrams per hour, for 100 seedlings. The total amount of CO_2 produced by each culture was ascertained for each observation interval in each experiment period by absorbing the CO_2 in a measured quantity of standard $Ba(OH)_2$ solution, in an apparatus with some new features, and titrating the excess $Ba(OH)_2$ with standard HCl solution at the end of the interval. Observations on growth were made also and a numerical index of shoot elongation was secured from measurements made at the end of the entire experiment period.

PROGRESSIVE INCREASE IN CO_2 PRODUCTION WITH TIME.—The average rate of CO_2 production was generally greater for later observation intervals than for earlier ones in the same experiment. A number of exceptions to this general rule receive special attention. They are mainly confined to differences between rates for the first and second intervals and to combinations of temperature of 20° or below with oxygen percentages of 16 or below. Neglecting the exceptions, the increase in CO_2 production as time went on was generally slow for combinations of low temperature and low oxygen pressure, more rapid for combinations of low temperature and high oxygen pressure, and still more rapid for combinations of high temperature and high oxygen pressure. This acceleration in the rate of CO_2 production was most pronounced for the combination of 30° and an oxygen percentage of 90 or 95. To illustrate the increase in the rate of CO_2 production throughout the five intervals the following representative series of average hourly rates may be useful:

TEMPERATURE-OXYGEN COMBINATION	1ST INTERVAL	2ND INTERVAL	3RD INTERVAL	4TH INTERVAL	5TH INTERVAL
10°, 20 per cent.	0.39	0.41	0.45	0.53	0.61
20°, 20 per cent.	0.82	0.84	1.07	1.33	1.67
20°, 95 per cent.	1.02	1.38	2.20	3.60	5.01
30°, 95 per cent.	1.87	2.55	4.54	6.18	6.87

Special attention is given to the manner in which the rate of CO_2 production increased with the progress of the experiment when the seedlings were subjected to the temperature-oxygen combination of 20° and 20 per cent., a combination probably frequently encountered in nature. The average rates shown under these conditions for the five intervals are respectively: 0.82, 0.84, 1.07, 1.33 and 1.67 mg. and the acceleration is seen to be almost uniform. The rate is shown to have increased by about 0.025 mg. per hour during the 33-hour period between about the 9th hour and about the 42nd hour.

NO MINIMAL OR MAXIMAL COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE FOR CO_2 PRODUCTION IS SHOWN.—No culture failed to give measurable CO_2 production with any tested combination of temperature and oxygen pressure in any interval and consequently no minimal combination or maximal combination is shown for any interval or for the whole experiment period. The lowest rate for every interval is for the combination of 10° and an oxygen pressure of 0.6 per cent., which gave rates of 0.26, 0.27, 0.28 and 0.29 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively. The combination of the highest temperature tested (30°) with the highest oxygen pressure tested (98.3 per cent.) gave high average rates in all intervals. These averages are 2.42, 4.07, 5.47 and 6.38 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively.

OPTIMAL COMBINATION FOR CO_2 PRODUCTION.—The optimal combination of temperature and oxygen pressure for CO_2 production in the several intervals is shown as the combination of 30° and 90 per cent. (first three intervals) or 95 per cent. (last two intervals). In no case did the optimal combination include the highest oxygen pressure tested (98.3 per cent.). The highest rates shown for the second, third, fourth and fifth intervals are, respectively, 2.88 mg. (for 30° and 90 per cent.), 4.65 mg. (for 30° and 90 per cent.), 6.18 mg. (for 30° and 95 per cent.) and 6.87 mg. (for 30° and 95 per cent.).

RANGE OF POSSIBILITIES WELL REPRESENTED BY TEMPERATURES AND OXYGEN PRESSURES EMPLOYED.—The graphs and tables show that the 5-degree temperature intervals used in this study might have been narrower without the introduction of confusion due to unexplained variation among the different lots of seedlings, and some of the wider intervals of oxygen pressure might also have been reduced in width. On the whole, however, the intervals chosen for sampling the logical possibilities of combinations of temperature and oxygen pressure proved to be satisfactory for such a survey as this.

ISOPLETHS FOR EQUAL RATES OF CO_2 PRODUCTION IN THE SECOND INTERVAL.—The temperature-oxygen relations of CO_2 production in the second observation interval are set forth by a system of isopleths in a solid diagram and also by a set of plane graphs. The diagram shows clearly how the same rate of this process may result from many different combinations of temperature and oxygen pressure. For the region of this diagram between the lines for oxygen pressures of about 20 and about 90 per cent. the relation between temperature and oxygen pressure is shown to have been approximately linear for any rate value and the proportionality is about the same for all rates above 1.0 mg. Within this range the rate of CO_2 production for any oxygen pressure is shown to have been generally higher with higher temperature and the rate for any temperature was generally higher with higher oxygen pressure.

DOUBLE OPTIMAL OXYGEN PRESSURES FOR CO_2 PRODUCTION.—For the several intervals and also for the entire period two optimal oxygen pressures for CO_2 production are generally shown with each temperature, these being the abscissae for two distinct graph maxima. Between the two graph maxima there is of course a subordinate graph minimum. The first or subordinate optimal oxygen pressure and the pressure corresponding to the second or subordinate graph minimum are generally higher with higher temperatures; they generally vary from about 6.3 per cent. and 9.8 per cent. (for 10°) to about 16 per cent. and 20 per cent. (for 30°). The second or main optimal oxygen pressure is generally shown as 90 or 95 per cent. The physiological meaning of the double optimum of oxygen pressure for CO_2 production, a feature apparently not previously mentioned in the literature of plant respiration and one that seems to be important, may be illustrated by a concrete example from the data for 25° and for the whole experiment period, as follows: The oxygen pressure of 9.8 per cent. (first or subordinate optimum for 25°) gave a much higher rate of CO_2 production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. were about alike. And a much higher rate was given by 95 per cent. (main optimum for 25°) than by 9.8 per cent. The totals for these critical pressures, for 25° and for the entire period, are shown below.

Lowest oxygen pressure tested (0.6 per cent.)	46.0 mg.
First or subordinate optimal pressure (9.8 per cent.)	98.4 mg.
Second or subordinate graph minimum (20 per cent.)	80.4 mg.
Second or main optimal pressure (95 per cent.)	190.8 mg.

Neither for any of the observation intervals nor for the whole period did the highest oxygen pressure tested (98.3 per cent.) give the highest rate

of CO_2 production for any temperature, and the pressure 98.3 per cent. was consequently supra-optimal for CO_2 production in every instance.

PROPORTIONALITY OF CO_2 PRODUCTION TO OXYGEN PRESSURE.—As in the case of the second and later intervals, CO_2 production in the whole period is shown for any temperature as about proportional to oxygen pressure within the pressure range from that for the subordinate graph minimum to that for the main maximum; *i.e.*, between about 20 or 30 per cent., and about 90 or 95 per cent. The constant of proportionality is shown to be greater with higher temperature.

CARDINAL TEMPERATURES FOR SHOOT ELONGATION.—The data for shoot elongation in the entire experiment period are discussed in detail and their relations are compared to the corresponding relations of CO_2 production. No main minimal temperature is indicated for shoot elongation; it was clearly below 10° for every oxygen pressure tested. On the other hand, a minimal oxygen pressure for measurable shoot elongation in the experiment period is shown for every temperature tested and this critical pressure was progressively greater with higher temperature. The lowest pressures giving measurable shoot elongation were: for 10° and 15°, 6.3 per cent.; for 20°, 9.8 per cent.; for 25°, 15 per cent.; for 30°, 20 per cent.

DOUBLE OPTIMAL OXYGEN PRESSURES FOR SHOOT ELONGATION.—The graph for shoot elongation with each temperature generally shows three low or minimal regions and two high or maximal regions, when oxygen pressures are abscissae and indices of shoot elongation are ordinates. It is therefore necessary to consider a double optimum of oxygen pressure for each temperature, as in the case of CO_2 production, but the details are very different for the two processes. Such a double optimum of oxygen pressure seems not to have been recorded in the literature of plant growth and it appears to be important.

The first graph maximum for shoot elongation corresponds to a pressure range (first or lower optimal pressure) of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°, to a pressure range of 30–50 per cent. for 20° and to a pressure of perhaps 75 per cent. for 30°. The second or main graph maximum corresponds to a pressure (second, upper, or main optimal pressure) of 90 or 95 per cent. for every temperature. The intervening second or subordinate graph minimum corresponds to a pressure of 75 per cent. with temperature of 15°, 20° and 25° and to a pressure of 90 per cent. with 10° and 30°.

It is remarkable that, for every temperature tested, the first or main minimal oxygen pressure for shoot elongation is shown as coincident with

the corresponding first optimal pressure for CO_2 production. CO_2 production in the whole period was regularly more rapid with higher oxygen pressure up to the pressure that just allowed measurable shoot elongation, beyond which CO_2 production was notably lower with still higher oxygen pressures until the subordinate minimal region of the CO_2 graph is passed. Although a considerable rate of CO_2 production may be supposed to have been necessary for growth, yet the occurrence of growth may have retarded CO_2 production, as might be true if the two processes were competing for the same plastic materials.

The highest oxygen pressure tested (98.3 per cent.) was supra-optimal for shoot elongation, as well as for CO_2 production, with every temperature employed.

EFFECTIVENESS OF TEMPERATURE AND OXYGEN PRESSURE IN DETERMINING CO_2 PRODUCTION AND SHOOT ELONGATION.—The relative magnitudes of the temperature influence and the oxygen influence on the rate of CO_2 production and shoot elongation in the whole experiment period are examined by means of coefficients of effectiveness for environmental differences. Within the range of temperature-oxygen combinations studied, the temperature influence was much greater than the oxygen influence with respect to both processes, and both influences were more pronounced with respect to CO_2 production than with respect to shoot elongation. The two influences were generally synergistic when operating together; *i.e.*, the effect of a temperature difference and a simultaneous oxygen-pressure difference was greater than the summation of the effects of the two differences considered separately.

CARDINAL TEMPERATURES FOR CO_2 PRODUCTION AND SHOOT ELONGATION COMPARED.—The main cardinal values (minimum, maximum and optimum of temperature and of oxygen pressure) for CO_2 production and for shoot elongation in the whole experiment period, are studied and the two processes are compared with respect to these values. The minimal temperature for both processes was below 10° for every oxygen pressure for which data are available. The maximal temperature for CO_2 production in the whole period is shown as above 30° for every oxygen pressure tested, while for measurable shoot elongation it varied directly with the oxygen pressure; this maximum is shown as between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 16 per cent., somewhat above 30° for pressures of 20–98.3 per cent. and farthest above 30° for a pressure of 95 per cent. The optimal temperature for CO_2 production was actually above 30° for every oxygen pressure tested but for shoot elongation this optimum varied directly with the oxygen pressure in the lower portion

of the pressure range. It is shown as 10°–15° for a pressure of 6.3 per cent., 15° for pressures of 9.8 and 16 per cent., 20° for pressures of 20 and 30 per cent. and 25° for pressures of 50–98.3 per cent.

MAIN CARDINAL OXYGEN PRESSURES FOR CO_2 PRODUCTION AND SHOOT ELONGATION COMPARED.—There is probably no such thing as a main minimal oxygen pressure for CO_2 production with any temperature, but for measurable shoot elongation in the experiment period the main minimal oxygen pressure is shown to have varied with temperature, being higher with higher temperature, as has been mentioned. The main maximal oxygen pressure for each temperature is shown as above 98.3 for both processes and attention is called to the consideration that an oxygen pressure higher than 100 per cent. would be impossible of attainment unless the total gas pressure were above 1 atmosphere. With respect to the main optimal oxygen pressures, as has been noted, the two processes differed very much. For CO_2 production this main oxygen pressure optimum was 90 per cent. with 10° and 30° and 95 per cent. with 15°, 20° and 25°, but it varied with temperature in quite another manner for shoot elongation.

The optimal combination of temperature and oxygen pressure for CO_2 production in the whole period is shown as the combination of 30° and 90 per cent. while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and 95 per cent.

It is remarked that the highest rates of CO_2 production for the entire period and the corresponding total shoot elongation probably approximate the capacity of these seedlings for the given period. Thus, they had the capacity to develop shoots 12.2 mm. long by the end of the period if subjected to the optimal combination of temperature and oxygen pressure for this process, and they had the capacity to produce 232.6 mg. for 100 seedlings by the end of the period if subjected to the optimal combination for CO_2 production. These are physiological characterizations of the seedlings used in this study.

CARDINAL TEMPERATURES AND OXYGEN PRESSURES FOR CO_2 PRODUCTION IN THE SEVERAL INTERVALS.—With regard to the cardinal values of temperature and oxygen pressure for CO_2 production in the several observation intervals, no minimal or maximal temperature is shown for any oxygen pressure in any interval. For all intervals, as well as for the whole period, these critical values are shown as respectively below 10° and above 30° for every oxygen pressure tested. As in the entire experiment period, no optimal temperature is shown for any oxygen pressure in any interval, and this value is indicated as above 30° in all instances, but that temperature was surely supra-optimal for health. No main minimal oxygen pressure is shown for

any temperature in any interval but this value was surely below 0.6 per cent. in every case, which is also true of the whole period. As has been noted, it is probable that there is no such thing as a main minimal oxygen pressure for CO_2 production with any temperature for seedlings such as these in any phase of their development. No main oxygen-pressure maximum for CO_2 production is shown for any temperature in any interval, but this was clearly above 98.3 per cent. in every case, just as for the entire period. On the other hand, the main optimal oxygen pressure for CO_2 production varied not only with temperature (as shown for the whole period) but also to some extent with the developmental phase of the plantlets. This main pressure optimum is generally shown as 90 per cent. for the first three intervals, and 95 per cent. for the last two intervals for all temperatures tested.

A FUNDAMENTAL PRINCIPLE EMPHASIZED.—The results of this whole study illustrate the most fundamental consideration for all research on the natural determination of process rates; namely, that the relation of any process to any influential condition or influence cannot be definitely stated without quantitative reference to the other prevailing influences. This is of course because of the inevitable concomitancy and interaction of all the influential conditions of the current environmental complex, including the time or duration factor. This general principle, which is basic for all physiological and ecological analysis, has been emphasized by BLACKMAN (**4**) in the following words: "The way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when this process is being pushed towards the upper limits of its activity."

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RELATION OF INCREASED WATER CONTENT AND DECREASED AERATION TO ROOT DEVELOPMENT IN HYDROPHYTES

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(WITH ELEVEN FIGURES)

The dependence of root development of most plants upon aeration is clearly shown by water-logging the soil. In a few days, the usual cultivated plants turn yellow, show wilting, and may ultimately die. If the water is kept well aerated, plants may survive though submerged for weeks. Exclusion of oxygen from the roots of most plants interferes with the respiration of the protoplasm of their cells. This results in the death of the cells and consequently the roots fail to function as absorbers for the plant (**22**).

The importance of aeration in plant development has long been recognized by plant physiologists and a very large number of investigations have been made. In 1921, Clements (**7**) summarized nearly 700 such papers in his mongraph on "Aeration and Air-Content." The long-continued work of Cannon has added much to an understanding of this subject and his "Physiological Features of Roots" (**5**) includes the most extensive recent contribution to this problem. The importance of aeration in the production of crops and the growth of forests has been emphasized by the extensive researches of Howard (**15**), Howard and Howard (**16, 17**), and Hole (**14**) in India. A survey of the literature shows that, with rare exceptions, there is general agreement that the aeration of the roots promotes a better growth of tops. Most of the experiments were carried on in water cultures, *e.g.*, Hall, Brenchley and Underwood (**12**), Andrews and Beals (**1**), and Bergman (**3**), and some in soil, such as those of Hunter (**19**), Hole (**14**), Howard (**15**), and Knight (**21**). The response of the plant in most cases has been measured by the development of tops and little account has been taken, especially in soil cultures, of the organs directly affected by the increased aeration. The work of Balls (**2**), Hunter (**19**), Hole (**14**), and the Howards (**16, 17**) are among outstanding exceptions. Cannon (**5**) measured the effect of inadequate aeration on root elongation and not on the development of the root system as a whole.

In devising a simple experiment for classes in plant physiology and ecology to illustrate the effect of aeration upon root and root-hair development, *Typha latifolia* was employed (**24**). It was selected because it grows naturally under a wide range of conditions, from water-logged soil to one that has a sufficiently low water content to be well aerated. Differences in root response under conditions of poor and good aeration were so marked

that three other characteristic and widely distributed swamp and swamp-margin plants were also employed and an extensive study of their root behavior begun. The use of large containers furnished an opportunity to determine the normal nature and extent of the underground parts, a procedure which met with almost insurmountable difficulties where the plants grow naturally in soil covered with water.

Procedure

Rhizomes of *Typha latifolia, Scirpus validus,* and *Spartina michauxiana* were dug in quantity on April 5, before new growth had begun. They were kept in a refrigerator for a period of 14 days before planting on April 19. *Phragmites communis* was taken from a swamp at this time and immediately transplanted. Growth was just starting.

Four large galvanized iron containers, 30 cm. square in cross section, were used for each species. The following conditions of soil moisture and concomitant aeration were maintained: (1) saturated, no free air; (2) alternately saturated and drained, aeration poor; (3) optimum water content for land plants, aeration good; and (4) dry soil, aeration good. The depth of the containers necessary to accommodate maximum root growth was determined by preliminary experiments; those for conditions 1 and 4 were 60 cm. deep, the others 90 cm. A well screened, fertile, loam, potting soil was used. It was mixed with one-third of its volume of sand. This mixture had a hygroscopic coefficient of 10 per cent. and a water holding capacity (HILGARD method) of about 55 per cent.

In filling the saturated containers, the soil was thoroughly saturated and constantly stirred to permit the escape of air. After the soil had settled, an excess of water was added to the container so that a layer of clear water 2.5 to 5 cm. deep stood constantly above the soil. Losses by evaporation were replaced every two or three days by adding tap water from a sprinkler. Six liters of water were daily poured on the gravel mulch of the drained soil. The water slowly sank through the soil mass and disappeared from the surface after 10 to 30 minutes. It slowly trickled through 2.5 cm. of coarse gravel placed on the bottom and out through an opening on the side. Here it was caught in a container and again used. This prevented loss of nutrients or other substances from the soil by leaching. Since there was some loss by evaporation, fresh tap water was added from time to time as needed. The third set of containers had soil of optimum water content (25 per cent. based on dry weight) which was lightly tamped in filling. The soil was covered with a fine gravel mulch, as was the case with all the containers except the first lot. Only enough water was added to replace that actually lost by transpiration and surface evaporation. The dry con-

tainers were three-fourths filled with a dry soil which had only 12 per cent. water content. This was but 2 per cent. in excess of the hygroscopic coefficient. Above this was placed a 15-cm. layer of soil with a water content of 25 per cent. Only enough water was added to the upper soil to insure continued growth.

Before planting, the rhizomes were carefully selected and cut in such a manner that they were quite uniform in size, number of sprouts, etc. All of the roots were cut close to the rhizome and seven rhizomes were planted at depths of 3.75 to 7.5 cm. (varying with the species) in each container.

FIG. 1. Development of plants 17 days after transplanting the rhizomes. The sequence from left to right is dry, saturated, moist, and drained soil.

All of the plants, except *Phragmites,* rapidly developed. The growth of the reed, although transplanted when still young, was somewhat checked compared with the development of plants in the swamp (fig. 1). For the sake of clarity each species will be separately considered.

Results

TYPHA LATIFOLIA

The development of the aboveground parts of *Typha,* at the end of the 35 days they were permitted to grow, is shown in table I.

TABLE I

GROWTH OF *Typha latifolia* ABOVE GROUND WITH DIFFERENT MOISTURE CONDITIONS

CONDITION FOR GROWTH→	SATURATED SOIL	DRAINED SOIL	MOIST SOIL	DRY SOIL
Maximum height, cm.	111.0	106.0	86.0	66.0
Average number of leaves	8.4	8.1	7.6	7.0
Average height of leaves, cm.	46.8	42.3	33.0	27.5
Average width of leaves, mm.	13.0	12.4	9.5	9.4
Total dry weight of tops, grams	16.2	19.9	12.2	7.2

An examination of the table shows that in every way development decreased with a decrease in water content. The dry weight is only an apparent exception, since in the wet culture only 5 plants grew, but there were 7 in each of the others, except the dry soil where one plant died. Here, as among the other three species, the plants in the moist and dry soils had a darker green color throughout their period of growth, due probably, to the more favorable conditions for nitrification and nitrogen fixation. In the saturated and drained containers the roots were readily freed from adhering soil particles and their dry weight was obtained. The dry weight of tops and roots was in the ratio of 5.8 to 1 in the saturated soil and 5.6 to 1 in the drained.

SATURATED CULTURE.—The root systems were examined on May 23–25 when the plants were five weeks old. Those in the saturated soil were very shallow and consisted of two distinct parts, namely, soil roots and water roots. All originated from a basal node of the stem about 6 cm. below the soil surface and none from the old rhizome. The rhizomes shriveled upon the removal of accumulated food and in several cases they were decomposing, although the soil was not sour. The soil roots, 64 in number on one of the largest plants, pursued courses at all angles between the horizontal and the perpendicular in such a manner as to more or less thoroughly ramify a hemisphere of soil with a radius of 15 cm. Eleven of the longest extended to 28 cm. depth.

These shining white roots were 1.5 to 2 mm. in diameter and very turgid and brittle, especially the distal 10 cm. Branches were entirely absent on most of the roots or occurred only very rarely. On others, slightly brownish in color and probably older, thread-like laterals arose at the rate of 1 to 8 per cm. (rarely 14 on the best branched parts). These were exceedingly variable in their distribution, frequently long portions of the roots being bare. Laterals were 1 to 5 cm. (maximum 10 cm.) long, and entirely unbranched but much more abundantly furnished with root hairs than the main roots where they were relatively sparse.

FIG. 2. *Typha latifolia* grown in soil covered with standing water, showing the sharp differentiation between soil and water roots. Photograph taken with root system under water in its natural position.

Approximately half of the root system (66 roots by actual count) consisted of water roots. These arose at a higher position on the node than most of the soil roots. While some ran obliquely upward, often at a 45° angle, many grew vertically and then turned at right angles after leaving the soil (fig. 2). Other roots ran horizontally about 8 cm. and then grew abruptly upward. The portions in the soil were like those described, but upon entering the water they greatly decreased in diameter and branched profusely, the distal part becoming extremely attenuated (figs. 3 and 4). They began to appear only 5 days after the rhizomes were planted and their growth was rapid, some reaching a length of 10 cm. in 3 days. By the expiration of 10–14 days all were greatly branched with laterals a centimeter or less in length. New roots continually appeared and branching increased throughout the period of growth. The older ones reached a maximum length of 30 cm. but many of them were only about one-half as long. When mature, they were branched to near the tips but there were no laterals of the second order. The very fine primary laterals, which occurred at the rate of 20 to 55 per cm., varied in length from 1.5 to 2.5 cm. and were en-

FIG. 3. Water roots of *Typha* about two weeks after transplanting the rhizomes.

tirely free from root hairs. These fine water roots and their very abundant branches enormously increased the absorbing surface in the aerated portion of the culture. Aside from frequent renewal of the water, an abundance of *Ulothrix* and *Mougeotia* increased the oxygen content. The former alga was so abundantly attached to the roots that it gave them a greenish hue.

DRAINED CULTURE.—The root system in the drained soil was much deeper seated although the roots were most abundant and most branched in the surface 9 cm., which was the best aerated. Here they formed a dense

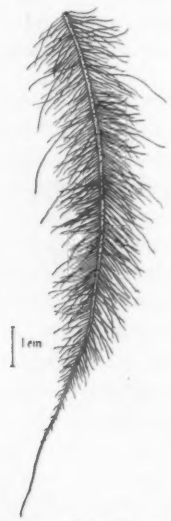

FIG. 4. Detail of water root of *Typha*. Note the attenuated tip and the profuse simple branching.

network. Roots were numerous to a depth of 30 cm. and some attained depths of 40 to 47 cm. (fig. 5). The general distribution of the roots was such as to occupy a more or less hemispherical mass of soil. They were rather clearly differentiated into three types as regards position, thickness, and degree of branching.

FIG. 5. *Typha* grown in drained soil. The finer, horizontal roots are near the soil surface.

One lot consisted of coarse, unbranched or practically unbranched roots which, as a group, penetrated deepest. Their diameters were 1.5 to 2 mm. throughout. A second lot was composed of roots with smaller diameters (1–1.5 mm.), some of which penetrated as deeply, or at least nearly as deeply, as the former. These branched profusely, often forming networks of laterals at depths of 30–36 cm. where branches 10 cm. long were found. The branching habit was variable; on some, only the proximal portion was

branched, on others, the distal part. On still others the presence of branches and especially their density varied from place to place. The rate of branching was 6 to 25 laterals per cm. The branches ranged in length from less than 1 cm. to a maximum of 10 cm., although most of them did not exceed 3 cm. Nearly all of the primary laterals were simple; a few were branched to the first order, the branches seldom exceeding 3 or 4 per lateral, and a centimeter in length.

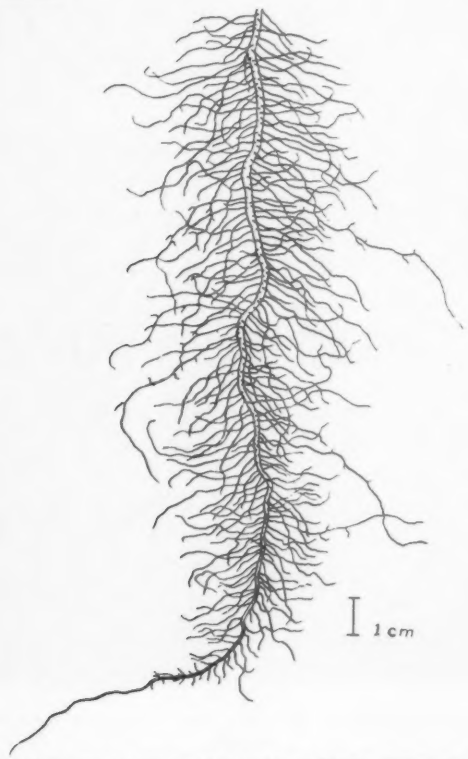

FIG. 6. Detail of one of the surface roots of *Typha* grown in drained soil.

A third type of root arose from the highest portion of the node at the base of the stalks and thus above the other roots. They thus originated at a depth of about 7 cm. and no portion of the root extended deeper than the point of origin. In fact, a few at first extended vertically upward but this tendency was not nearly so marked as in the case of water roots in the previous culture. Branches of these fine roots (which were 0.5 to 1 mm. thick) often occurred throughout, but in general they were most abundant and

longest on the distal one-third to one-half. Laterals were 7 to 10 per cm. on the more thinly branched parts and 12 to 20 on those most thickly branched. These thread-like roots were shining white, and the longest were more abundantly furnished with laterals than were any in the deeper soil. Secondary branches were few. The main root and all its branches were well supplied with root hairs. These roots differed from the water type by having longer, coarser, and more crinkly laterals with some sublaterals and an abundance of root hairs (fig. 6). The last was in striking contrast to the water roots, which had none.

One of the larger plants had 56 of the deep, unbranched type, 33 of the deeply penetrating branched ones, and 43 of the fine, surface type which were most favorably situated as regards aeration.

Moist culture.—The root system in the moist, well aerated soil showed no differentiation into parts; all of the roots were 1 to 1.5 mm. in diameter and about equally branched. There were no roots in the surface 7 cm. of soil. Long horizontal ones ran outward, sometimes to distances of 30 cm., but never turned downward. Others ran outward and downward to depths

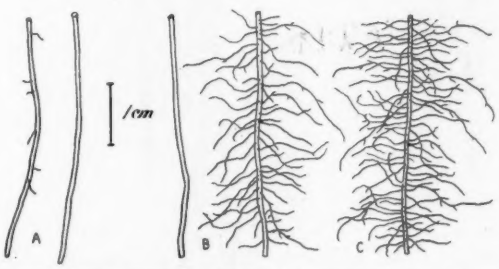

Fig. 7. Relative branching of roots of *Typha;* A, in saturated soil; B, in wet but drained soil; and C, in moist, well-aerated soil.

of 40 to 53 cm. All, except the shortest, were regularly branched except the rapidly growing, shining white distal 8 cm., upon which laterals had not had time to develop. Branches occurred at the rate of about 8 per cm. and varied in length from 1 to 2.5 cm. Secondary branches were only 1 to 3 mm. long and occurred sparsely and only on the longest primary branches (fig. 7).

Dry culture.—The root system was very much like that in the moist soil but more abbreviated. Its extent was determined by the depth of the moist soil which varied from 16 cm. on one side of the container to 35 on the other. None occurred in the surface 9 cm. (fig. 8). The dwarfed plants had fewer roots (27 and 51 respectively on two of the largest plants) than in moist soil but they were much more densely branched, *e.g.* 10–35 laterals

per cm., the longer ones having branches of the second order. The branches extended quite to the root tips where, owing to dry soil, further elongation was impossible. Where the tips had died, the roots gave rise to several long branches. In diameter, the main roots more nearly approached those of the moist soil than the thick roots of the wet soil (*i.e.*, they were mostly 1 mm. or less). They were more yellowish in color; root hairs were much more abun-

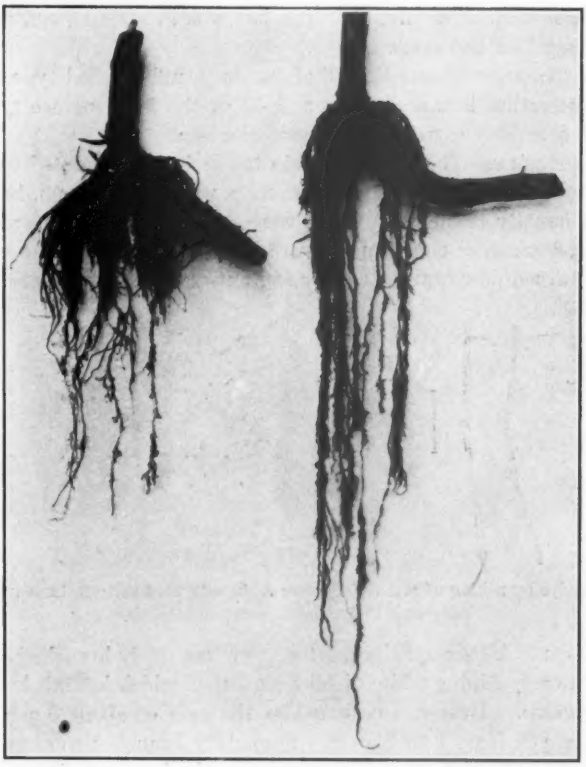

FIG. 8. Root system of *Typha* grown in dry soil. Short branches and root hairs are extremely abundant.

dant than in the drained soil, and, judging from the holding of the soil particles when excavated, more abundant also than in the moist culture. The root branches pursued markedly devious courses, in striking contrast to those in the wet and drained soils. One rhizome died without producing any growth, and another after some of its roots were only 10 cm. long.

RÉSUMÉ.—A survey of the preceding data shows that decreasing aeration on the one hand and decreasing water content on the other, has a marked effect upon root habit. In moist soil, the root system was uniformly developed throughout, spreading downward and outward below the level of the rhizome to a depth of 40–53 cm. In dry soil a similar root habit was found, although the number of roots on the dwarfed plants was fewer and root-hair development was more profuse and occurred to the root ends, whose further elongation was limited by the dry soil.

Where aeration was somewhat deficient in the alternately saturated and drained soil, the root system was somewhat shallower and differentiated into two distinct parts, one of these containing two types of roots. The surface root system consisted of a network of long, fine, profusely branched roots abundantly supplied with root hairs. This was confined to the surface 9-cm. layer of soil which was best aerated. The deeper part comprised both coarse and fine main roots, the latter only being much branched.

Where plants were grown in standing water, about half of the root system grew upward and developed into water roots. These were so fine and their primary branches so abundant that for their volume they presented a large absorbing surface for dissolved oxygen. They were destitute of root hairs. Similar root development has been observed in undrained marshes. The soil roots penetrated less deeply (30 cm.) than in the drained soil but were of the two types described for the latter. The chief differences were fewer, finer, and shorter branches that were more irregularly distributed, and a relative scarcity of root hairs.

SCIRPUS VALIDUS

Scirpus made a good growth even in the dry soil. Growth was again, with one exception, in direct proportion to water content as shown in table II.

TABLE II

GROWTH OF *Scirpus validus* ABOVE GROUND WITH DIFFERENT MOISTURE CONDITIONS

CONDITION FOR GROWTH→	SATURATED SOIL	DRAINED SOIL	MOIST SOIL	DRY SOIL
Total number of stems	47.0	42.0	25.0	18.0
Average height, cm.	87.9	100.4	71.0	49.6
Average basal diameter, mm.	8.1	7.8	5.2	4.7
Dry weight of tops, grams	39.6	42.4	18.8	7.0

The panicles of the aerated (drained) culture were more abundant and further developed than those in the saturated soil. This accounts for the greater height and also for the greater dry weight. No flowers had formed

in either of the other cultures, the tips of about one-third of the plants in each being dead. The ratio of dry weight of tops to roots was just the opposite of that of *Typha,* being 5.5 to 1 for the saturated soil and 7.4 to 1 in the drained soil.

SATURATED CULTURE.—At the time of root excavation, on May 29, the water-logged soil of the wet container had a distinctly sour odor. About

FIG. 9. Root development of *Scirpus validus* in water-logged soil covered with 5 cm. of standing water. The general root depth is about 38 cm.

half of the original rhizomes were still firm, the rest had decayed. The root system consisted of a glistening white mass of long fibrous roots that rather fully ramified the soil (fig. 9). All of these originated from the bases of

the new shoots or from new rhizomes and none from the old ones except from the part directly below the shoot. Roots were abundant to a depth of 38 cm. and a maximum depth of 46 cm. was attained. As in the case of the cattails, the horizontal and obliquely ascending roots of the surface soil were much finer and very much more branched than the deeper ones. While most of these originated from the upper portions of the nodes, the roots were not sufficiently distinct to separate into groups as in the cattails. Although a few water roots 4 to 5 cm. long were formed, a week after transplanting, this portion of the root system failed to develop. The network of fine surface roots with extremely fine laterals occurring at the rate of 3–14 per cm., developed only in the surface 2 or 3 cm. of soil. The coarser, deeper roots, 1 mm. or less in diameter, were usually sparingly branched for $\frac{1}{3}$ to $\frac{2}{3}$ of their length. The branches were simple and rarely exceeded a cm. in length. The laterals varied from 1 to 9 per cm. and in general they decreased with depth. While the main roots throughout the container were sparingly furnished with root hairs, most of the branches had none.

DRAINED CULTURE.—All of the rhizomes in the drained, unsoured soil remained firm and undecayed. As in the saturated soil, new rhizomes had made considerable growth, some a maximum of 7 cm. The root habit differed from the preceding in the following respects: the surface portion was very poorly developed, the deeper roots pursued a less devious course, and the depth of penetration was greater (fig. 10). Although some roots extended above their point of origin at the base of the shoot or rhizome, they were mostly short (10 cm. or less) and much less abundant than in the saturated soil. In fact, the surface 5 cm. of soil at a little distance from the base of the plant was only sparsely threaded with these fine roots.

In the deeper soil, the general root mass extended to 50 cm., roots were numerous at 55 cm. and a maximum depth of 59 cm. was attained. The younger roots usually reached lengths of 15 to 20 cm. before branching began and they possessed few or no root hairs. The older roots were usually branched throughout their proximal half and not infrequently almost to their tips. The branches were more numerous on the older roots, the number and length of branches being practically the same as that recorded for the saturated soil.

MOIST CULTURE.—The root system in the moist soil was much deeper than under either of the preceding conditions. Roots were very numerous at 64 cm. depth and a maximum depth of 71 cm. was attained. They originated only from the portion of the rhizome adjacent to the growing shoot, or from the base of the shoot. The soil below the level of the rhizomes was filled with a mass of roots, although they were not so abundant as in the drained soil. In general, their course was rather directly downward. Many main

roots in the upper soil were 10 cm. or less in length and the tips were dead. Short laterals, rarely exceeding 9 cm. in length, occurred regularly at the rate of 9–14 per cm. throughout the length of the main roots, except on the youngest 5–10 cm. of the root-ends. It appeared that the extremely abun-

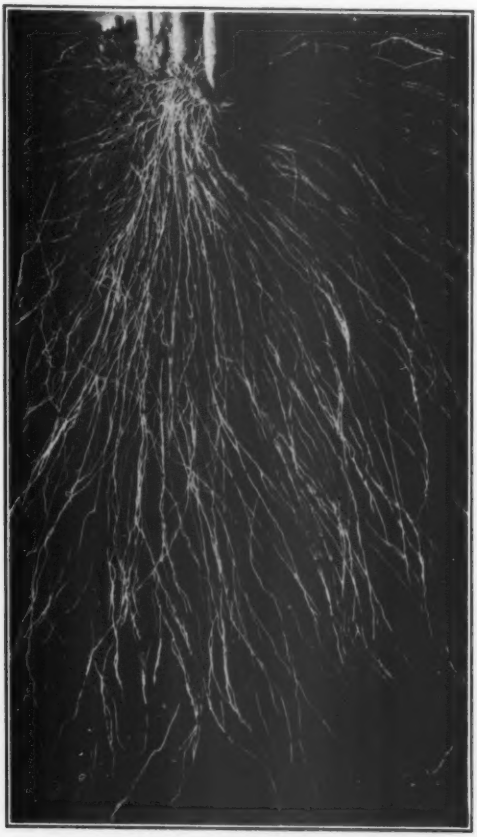

FIG. 10. Root system of *Scirpus* in drained soil (depth about 55 cm.). The development of a surface root system is much less marked than in figure 9.

dant roots were functioning throughout their entire course. Both the main roots and their branches were thickly covered with long root hairs. Portions of all of the old rhizomes showed decay and some were completely rotted.

DRY CULTURE.—The root systems in the dry soil were very much abbreviated as were also the tops. Roots were not only shorter but also fewer per plant, although more abundant in proportion to tops than in the wet soil. Insufficient water was clearly the limiting factor to growth since there was an abundant supply of food in the fairly well preserved rhizomes. As in the moist culture, there were many roots that extended horizontally or obliquely downward into the shallower soil but in no case were they found approaching the soil surface. The general direction of root growth was vertically downward or obliquely outward and then downward. The general level of root penetration was 30 cm., a few penetrating 10 cm. deeper. The older, deeper roots were profusely branched throughout but scarcely more so than in the moist container. Moreover, the branches were shorter, averaging only 3 mm. Since the main roots had ceased elongating, branches occurred to within 3 or 4 cm. of the tip. Although none of the primary laterals were rebranched, both they and the main roots were more densely clothed with root hairs than were those in the moist soil. Moreover, both branches and root hairs extended quite to the root tips. Hence, as shown in figure 11, the soil was held tenaciously.

RÉSUMÉ.—A comparison of the root behavior under the different environments shows, as in *Typha*, that under conditions of poor aeration there is a development of a fine, much branched network of surface roots and a consequent decrease in depth of penetration. The bulrush seems to emphasize the surface absorbing system somewhat less than the cattail. No water root system was formed and the surface system was less extensive, being confined to the first 3 cm. of soil. Moreover, in the drained soil it was very poorly developed. Here the deeper roots were straighter and penetrated about 12 cm. farther. Under both conditions branching was sparse and root hair development poor. In both the well aerated moist and dry soils, the surface root system failed to develop. Roots were 14 cm. deeper in the moist soil than in the drained, branching was quite profuse, and root hairs were abundant throughout. Relative to size of tops, the dry soil had the most roots. Their limited extent was compensated in part by the profuse development of short branches to the tips and by the very marked development of root hairs.

PHRAGMITES COMMUNIS

Phragmites made the poorest growth of all four species, a fact undoubtedly due to its being transplanted after growth began. The tops decreased in average height from 101 cm. in the saturated soil to 8 cm. in the dry soil where very little growth occurred. Total leaf production ranged in the same sequence from 61 leaves to only 4, notwithstanding the fact that the dry culture had the largest number of stems. Leaf area ranged likewise

from 1,118 to 20 square cm., that of the drained soil exceeding the saturated. The dry weight of tops of the drained soil was also somewhat greater. The ratio of tops to roots was less in the drained than in the saturated soil.

SATURATED CULTURE.—When the container was opened for root study on June 3 it was found that the soil had a distinctly sour odor but none of the rhizomes were decayed. The root system was composed of two distinct parts, a very much branched surface system and a portion that would ultimately penetrate deeply. The roots of the latter generally originated from

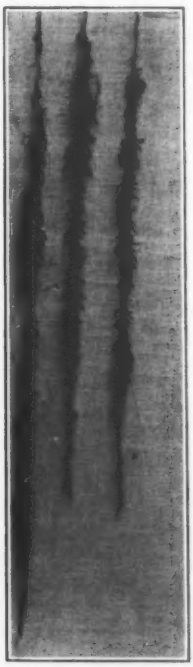

FIG. 11. Main roots of *Scirpus* grown in dry soil. Branches and root hairs occur to the root-tips.

the base of the rhizome branches, those of the former on the higher nodes of the shoot. Because of the repeated branching of the rhizomes at different levels, horizontal roots often originated more deeply than those that penetrated more or less vertically.

Some of the roots of the surface system arose as deep as 14 cm. and the surface soil was filled to its contact with the water with a mass of shining white rootlets. All were a mm. or less in thickness and none exceeded 28

cm. in length. While the younger ones often had unbranched ends several centimeters long, the older ones were profusely branched nearly to their tips. The branches were so very numerous (30 to 85 per cm., although only 0.5 to 10 cm. long and poorly rebranched) that the total surface for the absorption of water and oxygen was greatly increased.

The deeper roots had twice the diameter of the shallow ones. They extended to a depth of 21 cm. Only a few primary branches occurred and they were on the oldest part. Both main roots and branches of the entire root system were destitute of root hairs, except on a relatively small portion of the older ones.

DRAINED CULTURE.—The soil in this container was also sour but none of the rhizomes were decayed. With one exception, the depth of penetration was the same as before, but the following differences were plainly evident. The surface root system, although well developed, was not nearly so pronounced as in the saturated soil. Primary laterals on the entire root system were finer, longer, more numerous on the deeper parts, and more profusely branched, but rarely to the second order. Root hairs were abundant throughout the soil mass on both the main roots and practically all of the branches.

MOIST CULTURE.—The root system had made a poor growth in this container as had also the tops. Two rhizomes had died, one had not developed, and another had developed three short aerial shoots but no roots. The horizontal, surface root system consisted of wire-like, profusely branched roots, some occurring at a depth of 15 cm. That they were young was indicated not only by the absence of branches near the root-ends but also by the fact that approximately half of their laterals were simple. Moreover, they were not abundant. Of the few roots that penetrated downward, all but two were less than 16 cm. in length; the longest extended 34 to 37 cm. Root hairs were abundant throughout.

DRY CULTURE.—The poor growth showed that even the surface soil in this container was too dry for much development. Two rhizomes had decayed, three had developed short, erect stems but no roots, one had rooted and died. The others had from 3 to 6 roots each that varied from 1 to 20 cm. in length, but they were mostly less than 10 cm. The two types of roots were in evidence but neither was as well developed as in the preceding container.

RÉSUMÉ.—In the saturated soil, *Phragmites* developed a network of fine, extremely well branched roots which filled the surface 10 cm. to its contact with the water. The coarser, more or less vertically penetrating roots extended to about twice this depth and branched but little. There were no root hairs except on a small portion of the oldest roots. In the better

aerated, drained soil the root habit differed only in a less pronounced development of the surface root system and in the fact that root hairs were abundant on practically all of the main roots and their branches. In well aerated, moist soil the surface root system was represented by only a few horizontal roots, some of those of the deeper portion reaching nearly twice the depth attained in the poorly aerated culture. Few roots grew in the dry soil.

SPARTINA MICHAUXIANA

Spartina made a rapid growth, the plants in the saturated and drained cultures both reaching an average height of 75 cm., in comparison to 55 and 35 respectively for the drier soils. Dry weight of tops again decreased directly in the sequence of decreasing water content of soil except that the moist soil produced slightly more dry matter than the drained. The top-root ratios of the saturated and drained soils were 8.2 to 1 and 5.6 to 1 respectively.

SATURATED CULTURE.—The root system was more or less differentiated into a shallower and a deeper portion. The former consisted of a large number of fine, profusely branched, horizontally placed roots, some 20 cm. in length, that ramified throughout the shallower soil extending to its surface. Many of these ended without turning downward. They were branched abundantly with long laterals but root hairs were very sparse.

The deeper portion of the root system was chiefly composed of numerous coarse roots nearly 2 mm. thick that extended vertically downward or ran obliquely downward with little change in the direction of their course. Many ended at 28 cm. depth, a few extended 5 to 10 cm. deeper. Some of the shining white younger roots were only 10 cm. long, and entirely unbranched but sparsely clothed with root hairs. The last 10 cm. of the older roots was likewise frequently free from branches. Otherwise branching occurred at the rate of 9–16 laterals throughout. These varied in length from 1 to 10 cm. Root hairs were sparse and found on only relatively few branches. Short secondary branches occurred only on the longer laterals. Another lot of the deeper roots was much finer and could not be distinguished except by position from the fine surface roots. They were evidently older than those just described. All were abundantly clothed with fine laterals usually 0.5 to 3 cm. in length. Only the longer ones were rebranched and root hairs were sparse. Many new rhizomes had originated from the base of the clumps, but none exceeded 7 cm. in length.

DRAINED CULTURE.—The development of the rhizomes and general extent of the root system were very similar to those of the saturated soil, general root extent being only 5 cm. deeper. The surface portion of the root system was the same as in the previous culture but in neither were the roots densely

matted as in *Typha* and *Scirpus*. Not only was the rate of branching somewhat increased but also the primary branches averaged twice as long and fairly extensive laterals occurred even on the shorter rootlets. The roots were branched more nearly to their tips. Moreover, root hairs were much more abundant than in the saturated soil. Thus, the effects of increased aeration were clearly apparent.

MOIST CULTURE.—Quite in contrast to the abbreviated root system of the wet soil, 90 per cent. of the roots extended to the bottom of the container, thus having a length of nearly 90 cm. Moreover, none were found in the surface soil, all penetrating vertically or obliquely downward. Many of the coarse, rapidly growing roots were 2 mm. thick and the distal 15–25 cm. were unbranched. There was a decrease in thickness to approximately 0.5 mm. on older roots, and an increase in the degree of branching, long branches occurring close to the root-ends. Laterals occurred at the rate of 6–14 per cm.; branches exceeding 10 cm. in length were not abundant and most were shorter. The longest laterals (20–30 cm.) were found in the last 20 cm. of soil. Secondary laterals were common and root hairs abundant throughout. Where the roots entered the coarse gravel at the bottom of the container, their diameter was doubled and the branches were much coarser.

DRY CULTURE.—The roots had penetrated quite beyond the 20 cm. of moist soil and through the dry soil to the bottom of the container at 58 cm. In one case this was true of 14 roots from a single rhizome. In many cases they extended 5–14 cm. along the bottom of the container and gave off great clusters of long branches very nearly to their tips.

The roots were distinctly smaller in diameter than those in the wet soil, none exceeding a mm. and some were only $\frac{1}{3}$ as large. This was in striking contrast to the thick, rapidly growing roots of the moist soil.

All of the numerous coarse roots ran either vertically or obliquely downward so that there were none in the surface 6 cm. of soil. Branching was very similar on all of the roots, being uniform and profuse throughout the soil mass. It was somewhat more profuse than in the moist culture, and many branches, especially near the root-ends, were longer. Branches occurred at the rate of 9 to 22 laterals per cm. on both main roots and primary laterals. The primary laterals usually extended out horizontally 2–4 cm., although many, especially near the root-ends, were longer. Branches of the third order were not infrequent, all of the smaller branches being very fine.

RÉSUMÉ.—*Spartina* reached the same height in saturated and drained soils. Decrease in dry weight of tops accompanied decrease in water content, except that in the moist soil dry weight was slightly greater than in the drained.

The grass responded to poor aeration by the development of numerous fine, long, well branched horizontal roots extending to the soil surface. This

part of the root system was less poorly developed than in either *Typha* or *Scirpus*. The deeper portion of the root system was only slightly more extensive in the drained soil, but here it was furnished with more and longer branches. Root hairs were sparse in water-logged soil but more abundant in the drained.

No surface aerating roots developed under good aeration in moist soil, but the others grew to almost three times the depth attained in the intermittently water-logged soil. Branches were more numerous and root hairs very abundant throughout. Roots and branches in the coarse, well aerated gravel, were of much greater diameter. In the drier culture the roots penetrated soil of only 2 per cent. available water content, their extent being limited by the size of the container. Branching was most profuse here and the number of branch orders greatest. Root hairs were much more abundant also than in the moist soil.

Discussion

Of the four species investigated, three are characteristic of the reed-swamp stage of the hydrosere, all being very widely distributed. *Scirpus* grows in the deepest water, sometimes in excess of 6 feet, and *Typha* in shallower places. *Phragmites* usually occupies wet areas which are submerged at least for a part of the growing season, while *Spartina* represents a very late stage of the hydrosere and forms a transition between swamp plants and more mesophytic vegetation. Owing to changes in water level resulting from drought, flooding, and other causes, the above sequence is not absolute and relicts of cattails and bulrush often occur intermixed with the reed. In fact, the great extremes of water and air content endured by the plants, once they are thoroughly established, are indeed remarkable. *Spartina*, in these experiments for example, flourished in water-logged soil and also, after growth was started, made a fair development in soil with only 2 per cent. available water content.

These experiments show clearly that plants growing naturally in poorly drained and poorly aerated habitats are much less sensitive to the composition or absence of a soil atmosphere than are those in naturally well drained soils. In water-logged soil the oxygen supply must be very low or practically nil. Oxygen diffuses slowly through water, so that the supply is not quickly replenished by diffusion alone. The addition of tap water through a sprinkler to that standing on the soil surface together with the photosynthetic activity of the algae considerably increase the supply of dissolved oxygen. It has been demonstrated by several investigators that algae may bring about the supersaturation of lakes and streams as a result of photosynthesis, at the same time preventing the accumulation of large amounts of carbon dioxide, in consequence of the same process (**3, 13**).

All four species showed a distinct aerotropic response by the development of a superficial root system with a very extensive absorbing area. *Typha* was the only species, however, that extended its roots into the water layer above the soil. This development of an aerating portion of the root system and its absence under conditions of good aeration is in accordance with the findings of WILSON (**26**). He observed that the number and size of "knees" of the bald cypress (*Taxodium distichum*) were determined by the height of the water and the duration of the flooding. Young roots often grew directly upward until they reached the surface when they again turned and developed beneath the water. In dry soil the trees showed no trace of such development. Similar structures occur about the bases of trunks of Tupelo gum (*Nyssa aquatica*) in swamps, the roots bending sharply upward to a distance of 6 to 8 inches above the surface of the water and then turning downward into it again.

The production of adventitious roots which run horizontally above oxygen-free swamp soil, as in *Alnus, Fraxinus,* and various other land species, is well known (cf. **8**). But less exact information is available on the root behavior of true hydrophytes, although upright roots have been developed on *Rumex* and *Nymphaea* when these were planted too deeply (**11**).

In considering root response to aeration it must be kept in mind that species vary widely, a substratum of water or water-logged soil may furnish sufficient oxygen for certain hydrophytes, while under similar conditions most plants would succumb. CANNON (**4**) has shown that the temperature relation is of great importance in problems of aeration, the species studied demanding better root aeration as the soil temperature became higher. Since these cultures were grown under a moderate and rather uniform soil temperature (about 70° F.) the temperature factor need not be considered. Although there is some evidence that the rate of root respiration in hydrophytes is lower than that of land plants (**10**), the capacity of marsh plants to grow in water-logged soil is undoubtedly due to their extensive development of aerenchyma in leaves, stems, and roots (**25**). The continuously open stomata in many of them (*e.g., Typha* and *Scirpus*) would facilitate rapid gaseous diffusion. The extent to which aerenchyma develops when the roots of grasses like *Spartina* are continuously submerged remains to be determined, although a marked increase of intercellular spaces in roots of corn, etc., under such conditions has been clearly shown (**20**). The dependence of root-hair development of various plants upon the presence of oxygen has been pointed out by several investigators (**6, 23**). Although water roots are commonly without hairs, they may readily be produced on some plants in an aqueous medium provided that calcium is present (**9**). Root elongation in nearly all plants is likewise retarded by a deficient oxygen supply.

Summary

Plants of great bulrush (*Scirpus validus*), cattail (*Typha latifolia*), reed (*Phragmites communis*), and tall marsh grass (*Spartina michauxiana*) were grown in large containers under four sets of conditions of aeration and water content, *viz.*; water-logged soil, soil alternately saturated and drained, moist soil, and dry soil.

Growth of tops in *Typha*, as measured by number and size of leaves and total dry weight, increased with increasing water content until a condition of saturation was reached. More and larger *Scirpus* grew in the saturated soil but because of earlier flower-stalk production in the drained soil, both height growth and dry weight of tops were greatest in that culture. Both *Phragmites* and *Spartina* grew about equally well in the water-logged and drained soils but like *Typha* and *Scirpus* they became progressively poorer as the soil became drier.

The ratio of tops to roots (based on dry weight) was less in the drained than in the water-logged soil, except in *Scirpus*.

Typha alone had roots that developed in water. These were of small diameter and possessed so many fine laterals that they presented a large surface for the absorption of dissolved oxygen.

A response to poor aeration was shown in all four species by the development of a shallow root system of fine, much branched roots which presented a large surface area in proportion to volume. This did not develop in well aerated soil, except to a limited extent in *Phragmites*.

Aside from this surface portion of the root system, the development of lateral roots increased with an increase in aeration. Where dry soil hindered or prevented root elongation, branching occurred to the root tips.

Root depth increased with decreasing water content until the soil became too dry for root growth.

Root hairs were absent in water, few and irregularly distributed in saturated soil, but progressively more abundant as aeration became better with decreasing water content.

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A FURFURAL-YIELDING SUBSTANCE AS A SPLITTING PRODUCT OF PROTOPECTIN DURING THE RIPENING OF FRUITS[1]

C. M. CONRAD

Introduction

While studying the CARRÉ (**4**) procedure for the determination of protopectin, the writer observed that the filtrates from the calcium pectates contained a considerable quantity of a furfural-yielding substance. At about the same time EHRLICH'S (**9**) preliminary paper came to the writer's attention. In this paper EHRLICH showed that the protopectin (he calls it "pectin") of sugar beets and a considerable number of other vegetables and fruits is split by boiling water into two fractions—"a levo-rotatory araban," and "a calcium-magnesium salt of pectic acid." A mixture of the two, called "hydrato-pectin" is obtained by boiling the plant tissues, previously freed from sugars, with water and evaporating the extract to dryness on the water bath. The araban is then separated from the pectic substances by extraction with 70 per cent. alcohol. The work of EHRLICH (**11**) on sugar beets has been confirmed, in general, by SMOLENSKI (**20**) and his co-workers.

The exact nature and significance of EHRLICH'S work seems not to be very well appreciated by other investigators in this field. For example, MCKINNIS (**16**) apparently fails to distinguish between the extraneous "araban," soluble in 70 per cent. alcohol, and the arabinose contained within the pectin molecule (**17**). It is likewise not clear from DORE'S (**8**) discussion that he distinguished between the two. WICKMANN (**21**) found a furfural-forming complex in his pectic acid filtrates but ascribed it to disintegration of pectin. In spite of EHRLICH'S work, EMMETT (**12**) assumes that no alcohol-soluble substance forms from the insoluble cell wall material of pears during ripening. However, she is at a loss to explain a certain unidentified constituent in the juice which makes up 20 to 30 per cent. of the dry weight.

It should be noted that EHRLICH uses a different nomenclature from that generally used, and from that proposed by the Committee (**6**) on Nomenclature of Pectic Substances. Thus his "pectin" corresponds to the protopectin of that committee report and his "calcium-magnesium salt of pectic acid" to the pectin. This unfortunate difference in terminology is, no doubt, largely responsible for the present confusion and the failure to draw correct deductions from EHRLICH'S work.

[1] Approved for publication by the Director of the Experiment Station.

By boiling the tissue with water, EHRLICH brought about a splitting of beet and flax protopectin into pectin and a polysaccharide. Allowing for the different nomenclature he assumed the following reaction:

$$\text{protopectin} + H_2O \longrightarrow \text{pectin} + \text{polysaccharide}$$

The polysaccharide was composed of pentose only (sugar beet) or of pentose and hexose (flax stems, **10**). Since pectin increases naturally (**2**) during the ripening of many fruits, it was the purpose of this investigation to determine whether a pentose-bearing polysaccharide is liberated at the same time. A reaction taking place according to the above equation should show a definite relation between the amounts of pectin and polysaccharides for all stages of ripeness. Then by determining the furfural yield and noting whether or not the ratio of furfural to pectin is a constant it should be possible to test the correctness of the equation.

Preliminary experiments showed that the alcoholic extracts from ripe apples, bananas, and pears contained a considerable quantity of a furfural-yielding substance. A sample of Stayman apples contained substances soluble in 70 per cent. alcohol which yielded 0.67 per cent. of furfural based on dry weight. After acid splitting of the protopectin and extraction with 70 per cent. alcohol the same sample gave 0.57 per cent. additional furfural. When these values are calculated as pentose, they are equivalent to approximately one half the amount of the pectic substance. As neither unripe apples nor pears were available when the work was undertaken they were not studied in the present series. The first report deals with the occurrence of this alcohol-soluble furfural-yielding substance, and its relation to the pectic substances in several fruits during ripening. A later report will deal with the nature of the furfural-yielding substance.

Description of experimental material

The fruits studied were bananas, peaches, strawberries, and dewberries. They were either picked in different stages of ripeness, or taken at an unripe stage and allowed to ripen in a controlled temperature chamber at 30° C. This temperature had been found by previous work to bring about rapid pectic changes. The stage of ripeness was selected on the basis of color and softness.

BANANAS

Forty medium sized fruits were carefully cut from a bunch of green bananas and assorted into four lots of equal number and approximate weight. Lot I was sampled at once according to methods to be described. In this lot the fruits were so green that the skins could not be stripped off. The remaining lots were placed in the constant temperature chamber at

30° C. Lot II was removed after 2 days. The skins had yellowed considerably but the fruit was not ripe enough for eating. Lot III was removed after 7 days. The aroma was very noticeable and the condition excellent for the table. Lot IV was removed after 11 days. The skins had become dark and the fruits were in an overripe condition for the table.

Peaches

Early Georgia peaches, still firm to the touch, were obtained on the market. Four uniform lots of eight fruits each were selected. Lot I was sampled at once. The remaining lots were stored at 30° C. Lot II was removed after 19 hours. The flesh of the fruit was perceptibly softer than that of lot I. Lot III was removed after 46 hours. The flesh had become quite soft. Lot IV was removed after 72 hours. The skins were yellow, the flesh was very soft, and a marked aroma was present.

Strawberries

The variety Klondike, grown on the Experiment Station farm, was used for this experiment. Berries in three stages of ripeness were picked at the same time and a lot selected from each. Lot I contained fruits turning white just before coloring. The berries of lot II had colored over about half the surface. Lots III and IV were at the same stage and full ripe. Lot IV differed from lot III only in being stored for 47 hours at 30° C. During this period the berries had increased in color, withered considerably, and lost about 14 per cent. of their weight.

Dewberries

The berries in this case grew wild a short distance from the Station farm. Two lots were picked at the same time. In lot I the berries were just beginning to turn and were still hard to the touch. The berries of lot II were full ripe and soft.

Preparation and storage of the samples

Since the furfural-yielding substance was to be separated with 70 per cent. alcohol, and since this concentration is excellent for preservation, it was found convenient to use this medium for the storage of the samples. In case the fruit was ripened artificially the loss of weight during this process was determined. The weights of the pits in the peaches were noted and the loss of weight of this fruit was calculated as loss in the flesh only. The fruits were cleaned, pulped by means of a Nixtamal mill, and 100-gram samples of the pulp weighed out into 500-cc. Erlenmeyer flasks. The samples were covered with the calculated amount of 95 per cent. alcohol to give a final concentration of 70 per cent. by volume. At the same time moisture samples were taken to permit calculation to dry weight.

Separation and treatment of the different fractions

First, the furfural-yielding substance, soluble in 70 per cent. alcohol, was separated from the pectic substances by exhaustive extraction of the pulp. The storage alcohol was removed through a hardened paper on a Buchner funnel. The drained pulp was then washed with 200 cc. of 70 per cent. alcohol, added in successive 50-cc. portions. The pulp was drained by sucking as dry as possible before the addition of each new portion of alcohol. It was next transferred to a shaking bottle with 200 cc. of 70 per cent. alcohol and shaken for 30 minutes. This alcohol was drained off and the pulp again washed with 200 cc. of 70 per cent. alcohol, added in portions. The pulp, thus treated, contained no more furfural-yielding substance soluble in 70 per cent. alcohol until subsequently treated to decompose the protopectin. It was washed with two portions each of 95 per cent. alcohol and ether and dried at 30° C.

The free pectin, which was insoluble in 70 per cent. alcohol, was now extracted. The dried residues were first shaken an hour with 500 cc. of 0.2 per cent. ammonium citrate solution at laboratory temperature. The purpose of the ammonium citrate was to dissolve any pectic acid which might have been formed from the pectin by pectase activity. A previous experiment showed that shaking for 30 minutes brings about complete solution but it was continued for an hour to make certain. The extract was filtered off through soft fluted paper and an aliquot portion used for the pectin determination. The pulp was now further washed to remove the last traces of pectin. This was accomplished by washing back into the shaking bottles with 500 cc. of water or 0.2 per cent. ammonium citrate solution, in case any pectic acid was present, and shaking 30 minutes. The extract was filtered off and discarded. This process was repeated 5 to 7 times although not more than traces of pectin were ever found after the third washing. Finally the pulp was dried as previously described with 95 per cent. alcohol and ether.

There remained in the dried residues only substances insoluble in 70 per cent. alcohol and water,—among others, protopectin. The protopectin was decomposed by covering the residues with 100 cc. of thirtieth normal hydrochloric acid (in one case 150 cc.) and heating at boiling temperature under a reflux condenser for an hour. The mixture was diluted with sufficient 95 per cent. alcohol to give a final concentration of 70 per cent. The acid was then neutralized with the calculated quantity of standard sodium hydroxide solution. The polysaccharide and the liberated pectin were again extracted with 70 per cent. alcohol and then water exactly as described for the soluble fraction. The residues were finally dried and preserved.

Chemical methods

Moisture.—The samples, consisting of 3 to 5 grams of wet pulp, were dried to constant weight at 80° C. in a vacuum oven at approximately 2.5 cm. of mercury.

Determination of furfural from the alcoholic extracts.—The combined 70 per cent. alcoholic extracts and washings, amounting to something over a liter, were evaporated in a porcelain dish on the water bath to 30–40 cc. At this point any appreciable amount of sugar in the liquid was removed by fermentation. This was found to be necessary because of the production of hydroxy-methyl-furfural (**7**) on distillation of hexose sugars with 12 per cent. hydrochloric acid. The presence of this furfural derivative causes the development of a dirty green color in the FLEURY and POIROT (**13**) method instead of the pure blue due to furfural. The sugars were fermented away with washed baker's yeast, observing the precautions suggested by ABBOTT (**1**). The alcoholic extracts obtained in connection with the protopectin contained inappreciable amounts of sugars and were not submitted to fermentation.

The evaporated extracts, freed from more than traces of sugar, and separated from the yeast by filtration were transferred to a 750 cc. distilling flask. This was done by washing with sufficient concentrated hydrochloric acid and water to make 200 cc. of 12 per cent. hydrochloric acid solution. The mixture was then steam distilled and the furfural collected according to the procedure of PERVIER and GORTNER (**18**). Distillation was continued until ten drops of the distillate gave a negligible color with aniline acetate in acetic acid solution.

Because of the small amount of furfural in most of the distillates the usual method of determining the furfural by precipitation with phloroglucinol is unsatisfactory. Neither is the electrometric titration method of PERVIER and GORTNER (**18**) sufficiently sensitive. The bisulphate method of JOLLES (**14**) was tried but known amounts of furfural could not be recovered, quantitatively. The colorimetric method of SCHAFFER (**19**) was not very sensitive. The colorimetric method of YOUNGBURG and PUCHER (**22**) proved extremely sensitive and does not give any color with hydroxy-methyl-furfural, but due to instability of the color with these distillates checks could not be obtained. Finally the colorimetric method of FLEURY and POIROT (**13**) was selected because of its high sensitivity and satisfactory stability of color. In this method the colors obtained from the distillate are compared with those of standards prepared from pure furfural redistilled at low pressure. The following reagents are used:

Reagent A	
Acetic acid (pure)	1000 cc.
Orcine	0.625 gm.

Reagent B	
Hydrochloric acid (sp. gr. 1.19)	1000 cc.
Ferric chloride	0.06 gm.
Standard furfural solution	
Furfural, pure	1.0 gm.
Acetic acid	10.0 cc.
Water, to make	1000.0 cc.

The standard furfural solution contains 1 mg. of furfural per cubic centimeter. The solution has been found to maintain its strength over several months. More dilute standards are prepared by diluting this solution with 1 per cent. acetic acid solution. The concentration of furfural was checked by the standard phloroglucinol method.

The procedure for the FLEURY and POIROT method is as follows: Place 1 cc. (quantitative pipette) of the "unknown" furfural solution in a test tube. Immediately add 4 cc. of reagent A. In a second test tube place 1 cc. of the appropriate standard solution and add 4 cc. of reagent A to this, also. Next add to the tubes at one minute intervals, 5 cc. of reagent B. Plunge the tubes into a boiling water bath containing a "false bottom" and leave exactly one minute. Remove the tubes and allow to stand 30 minutes, when the color will have reached its maximum. The color remains at a maximum for at least 30 to 40 minutes more after which it may fade slowly. The manipulations of the colorimeter suggested by MCCRACKAN, PASSAMANECK, and HARMAN (**15**) were found to simplify calculations materially.

In using the FLEURY and POIROT method several precautions should be observed. The colors themselves cannot be diluted, so the "unknown" and standard colors should match within 20 per cent. If, on comparison, this limit is found to be exceeded a new colorimetric determination must be carried out with standard or "unknown" furfural solution diluted accordingly. Also reagent A should be made of a good grade of chemicals. The acetic acid must be free from furfural. This reagent should remain clear for a number of days. A dark solution, or one that turns dark quickly, will not give accurate color comparisons.

Pectin and pectic acid.—In bananas, this fraction was present entirely as pectic acid. In none of the other fruits was any pectic acid found. The pectic acid is assumed to have arisen from pectin through pectase activity. As stated above, the dried residues, after the first alcoholic extraction, were ground and shaken an hour with 0.2 per cent. ammonium citrate solution. After filtration, aliquots of the filtrate were taken for a determination of pectin by the CARRÉ-HAYNES (**5**) method. Impurities in the calcium pectates were evaluated by the method described by APPLEMAN and CONRAD (**3**).

Protopectin.—After complete removal of the soluble fraction with alcohol and water, as described in a previous section, the dried residues were boiled

an hour with thirtieth normal hydrochloric acid. This converted the protopectin into pectin and liberated more of the alcohol-soluble furfural-yielding substance. After separation of the furfural-yielding substance the residue was extracted with 0.2 per cent. ammonium citrate solution, or water, and the pectin determined as calcium pectate.

Experimental results

The results of the different experiments are brought together in table I.

As was to be expected, pectin increases and protopectin decreases during ripening. While the sum of the pectin and protopectin fractions is not entirely constant for the different stages of ripeness it nevertheless approaches a constant value and the discrepancy is possibly within the limit of the experimental error. Concurrently with the increase in pectin, there is an increase in polysaccharide as represented by furfural yield. The ratio of furfural to calcium pectate (column six) shows that the increase in polysaccharide is practically proportional to the increase in pectin, although this ratio seems to vary with the different fruits. In the protopectin fraction as protopectin decreases the polysaccharide decreases. Here, however, the ratio of furfural to calcium pectate is higher and fluctuates more, especially with bananas. A partial explanation for the higher ratios and greater fluctuation will be discussed in a later paragraph. The totals of calcium pectate and of furfural show a tendency either to increase or decrease progressively during ripening. This same tendency is shown by the ratios of the totals. However, the ratios here are more nearly the same for the different fruits.

Discussion of results

Inspection of the ratios of furfural to the pectic substances reveals a rather close relationship. Thus during the ripening of the different fruits, pectin and polysaccharide exist free in the sap in an approximately fixed proportion. However, this ratio varies from one fruit to another. It is possible that the protopectin itself may differ slightly in the different fruits, either in containing a greater proportion of polysaccharide or in containing a polysaccharide with a greater number of pentose units. The ratios in the protopectin fraction are, in general, higher. In bananas the ratio fluctuates greatly. However, the banana was the only fruit to show strong pectase activity and it is quite possible that pectinase existed here also and destroyed part of the pectin so that it was not determined. The high ratios of lots III and IV are undoubtedly due to low pectin figures and not to large amounts of polysaccharide.

A factor that makes all the ratios in the protopectin fraction higher than those of the soluble fraction is the destruction of pectin that accompanies

TABLE I

ANALYSIS OF FRUITS BASED ON ORIGINAL FRESH WEIGHT

Lot no.	Loss on storing	Moisture	Soluble in the fruit as sampled			From the protopectin by splitting with dilute acid			Totals by summation of the soluble and insoluble fractions		
			Polysaccharide as furfural	Pectin as Ca-pectate	Ratio: Furfural / Ca-pectate	Polysaccharide as furfural	Pectin as Ca-pectate	Ratio: Furfural / Ca-pectate	Polysaccharide as furfural	Pectin as Ca-pectate	Ratio: Furfural / Ca-pectate
	per cent.	*per cent.*	*per cent.*	*per cent.*		*per cent.*	*per cent.*		*per cent.*	*per cent.*	
					BANANAS						
I	0.0	72.75	0.003	0.106	0.03	0.015	0.201	0.07	0.018	0.307	0.06
II	5.13	73.17	0.012	0.295	0.04	0.014	0.061	0.23	0.026	0.356	0.07
III	15.90	79.94	0.012	0.387	0.03	0.011	0.007	1.56	0.023	0.394	0.06
IV	31.69	83.92	0.013	0.293	0.04	0.021	0.026	0.79	0.033	0.319	0.10
					PEACHES						
I	0.0	89.19	0.022	0.316	0.07	0.081	0.343	0.24	0.103	0.659	0.16
II	1.96	89.22	0.026	0.402	0.06	0.085	0.260	0.33	0.111	0.662	0.17
III	4.11	89.41	0.033	0.561	0.06	0.066	0.174	0.38	0.099	0.736	0.13
IV	6.02	90.00	0.040	0.560	0.07	0.043	0.130	0.33	0.083	0.690	0.12
					STRAWBERRIES						
I	.....	90.34	0.025	0.228	0.11	0.045	0.388	0.12	0.070	0.615	0.11
II	.....	91.13	0.031	0.319	0.10	0.020	0.160	0.13	0.051	0.478	0.11
III	0.0	93.05	0.040	0.319	0.12	0.018	0.081	0.22	0.058	0.400	0.14
IV	14.10	91.58	0.047	0.327	0.14	0.010	0.056	0.18	0.057	0.383	0.15
					DEWBERRIES						
I	.....	79.93	0.016	0.149	0.11	0.108	0.860	0.12	0.124	1.009	0.12
II	.....	84.04	0.021	0.506	0.04	0.053	0.195	0.27	0.075	0.701	0.11

boiling with dilute acid during hydrolysis of the protopectin. Certain experiments (unpublished data) have shown that 17 per cent. or more of the pectin may be destroyed during this period. Were such a correction applied, it would lower the ratios considerably, a number of them to approximately the same value as those from the soluble fraction.

Another factor which would no doubt affect the ratios is the variable amount of polysaccharide. In actual experiment using steam distillation it was found that pure arabinose gave 72 to 84 per cent. of the theoretical yield of furfural, depending principally on the amount of arabinose used. Since the polysaccharide must contain pentose this variable is most likely present.

Leaving the ratios of furfural to pectic substances, we may approach the problem from another angle. If the formation of pectin from protopectin is truly a hydrolytic process, and if the products do not undergo further decomposition, then the sum of pectin and protopectin all calculated as calcium pectate should be a constant for all stages of ripeness. In a like manner, the sums of furfural yields from the soluble and protopectin fraction should be the same for each stage of ripeness. Of course, this would only be apparent providing changes in the weight of the fruit during storage were ascertained and corrected for. Since this was done for bananas and peaches, it is possible in these cases to obtain a check upon this relation. Attention has already been called to the fact that these totals are not entirely constant. While for the pectic constituents there is less variation in peaches than in bananas, nevertheless, in both there is a progressive increase through the first three lots, followed by a decrease in the fourth. While the total furfural increases progressively in bananas it first increases and then decreases in peaches. A correction for loss of pectin during hydrolysis of protopectin nearly eliminates the fluctuation in the pectin totals for peaches but only partially for bananas. It may be that artificial hydrolysis of banana protopectin results in greater destruction of pectin than was found in other tissues. Of course these corrections would not account for the progressive changes in the total furfural. It is thus evident that during ripening, either metabolic changes or decompositions occur which obscure a mathematical verification, or the reaction is not strictly hydrolytic.

Summary and conclusions

It has previously been shown, and is here again substantiated, that during the ripening of fruits pectin increases at the expense of protopectin. It is now shown that concurrently with the development of soluble pectin there is liberated an unidentified furfural-yielding substance, soluble in 70 per cent. alcohol, which increases progressively with the pectin. It is also shown

that when protopectin is decomposed by boiling with dilute acid, more of the furfural-yielding substance is liberated. It is found in decreasing amounts as the amount of protopectin in the fruit is decreased during ripening. Although the ratios of furfural to the different pectic fractions are not entirely constant, the results seem to indicate that this furfural-yielding substance is a component part of protopectin, and is liberated by the splitting of protopectin during the natural ripening process. There is some evidence that certain metabolic changes take place in the pectic constituents themselves, during this period.

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EXPERIMENTS WITH *TRIANEA* ON ANTAGONISM AND ABSORPTION

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These experiments were undertaken in order to check by a different technique observations previously reported (**27**) in which seedlings of *Phaseolus sp.* and *Lupinus albus* were used. The following phenomena were observed: (1) that small concentrations of the Fe ion increased the growth rate of seedlings; (2) that the presence of the Ca ion inhibited, if it did not eliminate, the killing effect of certain otherwise lethal concentrations of Fe; and (3) that relatively high temperature (approximately 29° C.) increased the degree of permeability of the roots even though great amounts of Ca were present. The data to be presented here confirm the foregoing observations and present evidence indicating that Ca decreases the degree of permeability of cells to a marked extent.

General Considerations

All other plant requirements being normal, it is the opinion of the writer that the distribution of plants is a function of the Ca supply and of toxic substances, for the most part Fe and Al. That is to say, given a soil that can support a vegetation, the type that dominates or survives will be determined by the Ca, Fe and Al content of the soil colloids or soil solution. The pH of the soil or soil extract is believed to be a negligible factor (**26**) although it is a significant criterion of the concentration of ions to be expected on analysis of soil water (**1, 6, 13**). From a turning point at pH 5.0, Fe and Al are increasingly present in the soil extract, the concentration varying with the phosphate content as well as the pH of the soil solution (**1, 6, 13, 14, 25**). That some plants cannot survive a greater concentration than approximately 1.0 ppm. of Al or Fe has been shown in a recent paper (**27**).

It remains to consider why some plants require a relatively high Ca content in the growth medium while others are indifferent to the concentration of this ion; why some plants are so sensitive to the presence of Al and Fe, and why others are comparatively resistant. There are many observations bearing on these points.

NÄGELI (**16**) observed that when the calciphile *Achillea atrata* and the calciphobe *A. moschata* occur in the same valley, each is confined to its own soil type; but if either occurs in the absence of the other, it is non-discriminating. It is the opinion of SCHIMPER (**24**, see **22** and **23** for extensive references) that the failure of plants on chalk is due to the difficulty in

absorbing sufficient Fe, and that such failures may be obviated by watering with an iron solution. KERNER (**9**) has compiled a long list of contrasting plant species. Among them are *Rhododendron hirsutum* and *R. ferrugineum, Androsace pubescens* and *A. glacialis, Juncus hostii* and *J. trifidus.* The former of each contrasted plant pair is the calciphile, which, in order to survive, must have a soil which is relatively high in Ca. The latter of each contrasted plant pair is the calciphobe. Such plants either grow very slowly in a calcium rich soil or are indifferent to the presence of Ca. PAUL (**19**), in working with various *Sphagnum* species, found that those indigenous to high moors, where the salt supply is low, are normally much less resistant to Ca than those growing on the low moors where the minerals are more abundant. Less than 90 ppm. of lime were sufficient to cause death to the high moor *Sphagnum,* while over 200 ppm. were needed to kill the low moor species. The observations of SKENE (**28**) are of interest on this point—*Castanea* can grow on a chalky soil when supplied with abnormally large amounts of K, indicating that the chalk acts by interfering with the supply of other salts through the roots. He is of the opinion that "mineral solutions are generally physiologically harmless, but may be ecologically harmful." LOEB (**11**) records that $CaCl_2$ inhibits the twitching of muscles or nerves caused by Na_2SO_4, sodium citrate, or $N(C_2H_5)_4Cl$, which may be due to the prevention of diffusion through the membrane or to the action of Ca on protoplasm. Evidence points to the former view. In a later paper (**10**), he states that the diffusion of KCl through a membrane is due to a change in the external part of the membrane produced by the presence of other salts. Finally in a recent paper by HARDENBURG (**5**) the statement is made that "the increased applications of lime to lettuce reduced the growth of both tops and roots, decreased the total ash content and the absorption of Al, Fe, and Ca to a moderate extent."

Other citations are at hand but there is little to be gained by repetition. A great body of observation and data indicates most strongly that Ca decreases the degree of permeability of the plasma membrane with respect to substances entering the cell. Some plants find this essential (calciphiles) while others starve slowly or rapidly under the same conditions (calciphobes). This paper attempts to demonstrate the truth of LOEB's observation "that the external part of the membrane only is affected."

The various degrees of sensitivity of plants to Fe and Al seem to be coincident with their comparative indifference to Ca. This is largely conjectural as there are few data on the subject. From the preceding statements, the proof would necessitate the demonstration of variability in the plasma membranes (or, more conservatively, the protoplasm) of the plants considered. HANSTEEN-CRANNER's work (**4**) is most suggestive. He con-

cludes that there are water-soluble and water-insoluble lipoids making up the plasma membrane. Should this be the case, it would follow that those plants having small quantities of water-insoluble lipoids would require large amounts of Ca to maintain a water-in-oil emulsion at the free surface. A possible mechanism is explained by CLOWES (**3**). Contributions on the temperature relationships to the viscosity of protoplasm and to the permeability of the cell agree with this opinion (**4, 7, 12, 18, 27**).

The writer inclines toward this view but suggests a second, simultaneously operative. Even though the plasma membranes of root hairs and epidermal parenchyma cells are permeable, however slowly, to Fe and Al (as well as other cations, both toxic and relatively harmless), the cells of certain plants are not affected. This apparent indifference may be due to the precipitation within the cell of the Fe and Al ions as insoluble complex salts or esters, by ions already present within the cell and harmless to the cell in the quantities present. Two such kinds of compounds are probable, tannins and oxalates. The presence of the former in cells in significant quantities has been known for decades. MOLISCH (**15**) has ascertained the presence or absence of oxalates in 246 plant species.

This paper shows that the presence of tannin, sodium oxalate and haematoxylin in the root hairs of *Trianea* eliminates the toxic action of Fe for a considerable period even though Fe enters the cell constantly.

Methods

IMMERSION

The root hairs of *Trianea bogatensis* were the material used. Under ideal conditions, the protoplasm of the root hairs streams for hours when the roots are immersed in distilled water. The rate is constant for any reasonable time interval taken. A root from a healthy plant was cut and immersed and irrigated with distilled water. The cyclotic rate was measured by means of a stop watch and a graduated micrometer eyepiece. The protoplasm is normally very mobile and liquid in appearance and its viscosity appears to be low. There are particles present which either are suspended in the protoplasmic streams or which make up the living material itself. These are easily designated and observed and they vary in size from about 2–6 micra in diameter. Sometimes, indeed, they are much larger, in which case they constitute a specific gel. The rate of movement is independent of size.

Many streams of protoplasm can be observed in simultaneous motion. Generally one main stream moves from the tip to the base of the hair while a stream on either side of the hair progresses in the opposite direction.

From time to time, new currents appear at the expense of those already present. It is most convenient to measure the time necessary for a given particle to travel 50 micra. From 8–12 careful measurements can be made in a five-minute interval. When including all moving streams, this gives a fair cyclotic average. The results obtained for any five-minute interval constitute the average speed for that interval and are so reported.

Determinations on the rate of cyclosis of the protoplasm of a hair in distilled water have been made over a period of three hours. For any given five-minute interval, the cyclotic rate has varied less than 10 per cent. in carefully grown and selected material. This being the case, it was considered important to take only sufficient determinations to obtain a reasonable estimate of the cyclotic rate of the chosen hair in distilled water. This rate is the basis of comparison for subsequent experiments using the same hair. The time interval chosen was 20 minutes. Many such experiments were made simply as checks after an experiment of long duration was completed.

After the rate of cyclosis of a given hair had been determined in distilled water, the same hair was irrigated with a solution of 10 ppm. of Fe (as the nitrate) and 20 ppm. of Ca (as the chloride). Hairs irrigated with this solution have been observed for two hours on several occasions. The rate of cyclosis does not vary significantly and remains practically the same as that recorded for distilled water. Therefore, it was thought necessary in check experiments to make observations only over a time interval permitting a reasonable estimate of the rate of streaming. For check experiments, 20 minutes again was chosen.

The same root was then irrigated with a solution of ferric nitrate containing Fe at the rate of 10 ppm. The rate of cyclosis was determined for five-minute intervals until the death of the observed hair.

Injection

The micro-manipulation method and micro-injection have been described by Chambers (**2**) and Péterfi (**20**). The Chambers micro-dissection apparatus was used in this work. With micro-pipettes with external measurements of 3 or 4 micra, water, calcium chloride (20 ppm. of Ca), ferric nitrate, haematoxylin, tannin, and sodium oxalate were injected into the base of the root hairs.

Only pipettes which penetrate the cell wall with the greatest ease should be used. Otherwise mechanical injury to the hair becomes a most important factor. A good injection, so far as the writer can determine, offers no significant mechanical injury as judged by the rate of streaming of the protoplasm and its general appearance. This point was determined in the

following way: Five different hairs were pierced but not injected at intervals of five minutes. The rate of streaming was determined for each hair before it was pierced, then for five minutes after piercing and finally a half hour after piercing. All continued normal over the intervals mentioned. In one hair, however, cyclosis ceased for two minutes after puncturing; in three hairs for 30 seconds; and in the fifth hair no period of suppression was observed. Distilled water has no lasting effect on the cyclotic rate or on the health of the cells when it is injected.

It is believed, therefore, that any results observed after injecting a substance into the hairs are due to the reaction of that substance on the cytoplasm of the hairs and to nothing else.

The solutions of the salts of Fe, Ca, and Ca + Fe were injected into two or more hairs on a single root and the root then irrigated with distilled water while the observations were made. The numerous uninjected hairs on the same root served as a very excellent multiple check to observed reactions.

When solutions of haematoxylin, tannin, and sodium oxalate were injected while the root was suspended in a hanging drop of distilled water, the root was put immediately on a slide and irrigated with a solution of ferric nitrate (10 ppm. of Fe). Observations were made using uninjected hairs as checks.

In all cases the base of the hair was punctured. The opening quickly healed with a gel of protoplasm forming a plug which almost immediately became a brown coagulum. Other effects are described separately.

Criticism of methods

The most serious objection to the material employed is the difficulty in reproducing the results recorded. The root hairs of *Trianea* are exceedingly delicate cells and their "degree of health" varies greatly. This work was begun in December, 1927, and in the following five months the immersion experiments were concluded. At the time, it was observed that temperature played a most important rôle in toxicity. In order to obtain a curve, the experiments were continued. Unfortunately, it was found impossible to reproduce results with the root hairs then obtainable. New stolons were rooted and experiments repeated with the new crop the following autumn, without success. In spite of many efforts, suitable material was not obtained until the spring of 1929, but it was found impossible to conclude the work before the advent of hot weather. In order to complete the task, it became necessary to grow the plants in the following manner. Healthy plants were rooted in heavy garden soil in deep glass containers. The containers were immersed in 6-gallon jars and the new

stolons permitted to project into and root in the surrounding water. The 6-gallon jars were put into tubs filled with water cooled to 16° C. ± 3°. This kept the surface inch of water occupied by the roots of the daughter plants at a temperature of 18° C. ± 2°. In three weeks, sufficient roots were available to continue work. The hairs thus obtained were resistant to the treatment described and with them the entire work was repeated. Consistent reproducible results were obtained. Similar stock grown simultaneously in tanks with the temperature at 24° C. ± 3° were entirely unsuited for immersion experiments because of the great sensitivity of the hairs.

Experiments, therefore, are here considered as individual cases demonstrating a consistent tendency but not necessarily the degree of that tendency.

With respect to the micro-injection experiments, little effort was made to be quantitative. It is impossible as yet even to estimate the quantity of liquid injected. One can always be certain of an injection by the observable, though slight, displacement of particles in or of the protoplasm.

In the immersion experiments, only the most resistant roots were employed, while in injection, because of the time factor, very sensitive plants were chosen.

Results

Immersion

Table I shows the results of two experiments in which root hairs were irrigated first with distilled water, and then with ferric nitrate in the concentration of 10 ppm. of Fe.

It is apparent that this concentration of Fe causes a most pronounced acceleration in the rate of cyclosis soon after it is added as compared with the rate recorded for distilled water. The rate of streaming gradually decreases and finally stops. The protoplasm gradually aggregates at the tip of the hair and, just before death, moves with pulsating jerks and quickly turns a decidedly brown color. The tip of the hair may or may not burst. Should death occur quickly, the tip perceptibly swells and the protoplasm is extruded with a peculiar pumping motion.

Table II presents data of two similar experiments. The roots were irrigated with distilled water for the time interval indicated, then with a solution containing 10 ppm. of Fe and 20 ppm. of Ca, and finally with a third solution containing only 10 ppm. of Fe. These experiments are typical of a large number of similar observations.

In the presence of Ca it is noted that Fe has no effect on the cyclotic rate within the limit of the experiments. As previously remarked, no change was observed when this part of the experiment was twice continued for two hours. As soon as Ca is removed from the irrigating liquid, the

TABLE I

CYCLOSIS OF *Trianea* ROOT HAIRS IMMERSED IN WATER, FOLLOWED BY FERRIC NITRATE

IRRIGATING LIQUID	INTERVAL OF OBSERVATION	CYCLOSIS, PER SECOND
	minutes	μ
Water	5	4.2
Water	10	4.1
Water	15	4.2
Fe, 10 ppm.	5	4.8
Fe, "	10	5.7
Fe, "	20	4.9
Fe, "	25	3.6
Fe, "	35	3.9
Fe, "	50	3.6
Fe, "	90	3.0
Fe, "	100	No movement, brown, granular coagulum, death
Water	5	4.5
Water	10	4.2
Water	15	4.7
Water	20	4.4
Fe, 10 ppm.	5	4.5
Fe, "	10	5.5
Fe, "	15	5.3
Fe, "	25	7.3
Fe, "	35	5.0
Fe, "	40	5.1
Fe, "	50	3.9
Fe, "	55	Broke at tip, no movement, brown, granular coagulum, death

toxic action of Fe is exhibited in the increased rate of streaming, the following decrease, and characteristic death.

It is concluded that Ca antagonizes Fe in the concentrations used and that this antagonism is effected at the surface although not necessarily confined to the surface.

INJECTION

CA 20 PPM. (AS THE CHLORIDE).—When Ca is injected into the hair, a gel quickly appears in the region of injection and slowly becomes a coagulum. The rate of streaming decreases sharply until there is practically no movement of the protoplasm within 5 minutes. The gel is produced progressively from the point of injection at the base of the hair toward the tip, and death by the formation of a coagulum ensues in 10–15 minutes. Cells have been observed to produce a gel in about half the protoplasmic contents and then to convert it slowly into a sol, the entire hair becoming normal within 59 minutes after injection. Evidently this is a

TABLE II

ANTAGONISM OF CA AND FE ON CYCLOSIS OF *Trianea* ROOT HAIRS

IRRIGATING LIQUID	INTERVAL OF OBSERVATION	CYCLOSIS, PER SECOND
	minutes	μ
Water	5	5.5
Water	10	5.0
Water	15	5.3
Water	20	5.5
Ca, 20 ppm. + Fe, 10 ppm.	5	5.2
Ca, " + Fe, "	15	5.0
Ca, " + Fe, "	40	5.5
Fe, 10 ppm.	5	5.5
Fe, "	10	7.0
Fe, "	20	7.9
Fe, "	40	4.3
Fe, "	80	
Fe, "	85	Barely perceptible, no movement; brown, granular coagulum, death
Water	5	6.6
Water	10	6.0
Water	20	6.3
Ca, 20 ppm. + Fe, 10 ppm.	5	6.9
Ca, " + Fe, "	10	6.6
Ca, " + Fe, "	20	6.5
Ca, " + Fe, "	25	6.1
Fe, 10 ppm.	5	8.5
Fe, "	10	6.6
Fe, "	15	3.0
Fe, "	20	2.8
Fe, "	25	No movement; brown, granular coagulum; death

function of the quantity of Ca injected. Ca shows a decided toxic action on the hairs when injected, whereas no effect is observable when the hairs are immersed in much greater concentrations of the same ion (0.001 M.).

When compared with the reaction of the hairs on immersion in calcium chloride, the experiment implies that Ca is very slow in penetrating the cell; in fact, so slow as to be considered physiologically non-penetrating with respect to the criteria here used.

FE 10 PPM. (AS THE NITRATE).—Two types of results have been obtained with the injection of Fe, which the writer believes are simply functions of

the quantity of Fe injected into the cells. After injection the cyclotic rate is often increased similarly to the tendency reported in tables I and II for the immersion experiments. In other cases, the rate of protoplasmic streaming immediately decreases and the coagulation of the protoplasmic contents and the death of the cell occurs much faster than in the former instance. The sequence of events in the two types of reaction follows:

1. After injection, there is apparent a pronounced acceleration in streaming, beginning from 3–6 minutes after injection and continuing from 2–4 minutes. The rate then rapidly decreases, becoming almost imperceptible within 15 minutes. In upwards of 30 minutes the gel appears, which quickly becomes a coagulum. This coagulum is produced simultaneously in all parts of the hair. The protoplasm is tinted brown, and the hair is dead.

2. After injection, the streaming continues normal for perhaps 2 minutes, at which time the cyclotic rate decreases rapidly, becoming almost imperceptible within 10 minutes. A coagulum is formed simultaneously in all parts of the cell in from 15–20 minutes after injection, which rapidly becomes brown, and the cell is dead.

The toxicity of Fe when injected is patent. It causes a marked granular appearance of the protoplasm after death. The acceleration of the protoplasmic streams has been observed many times but the second method of death above described is more usual.

Ca 20 ppm. (as the chloride) + Fe, 10 ppm. (as the nitrate).—In no experiment was the rate of streaming observed to increase when this mixture was injected. On the contrary, the reaction of the protoplasm was apparently the same as though $CaCl_2$ alone were injected. However, the rate of cyclosis rapidly decreases and a gel is produced at the point of injection. This gel progresses toward the tip of the hair and progressively becomes a coagulum. Death occurs in from 10–15 minutes.

Injection and immersion

Sodium oxalate (saturated solution).—Root hairs were injected with this solution and then irrigated with ferric nitrate (10 ppm. of Fe). Table III shows the results of a typical experiment.

Immediately preceding the formation of the coagulum, a marked vacuolization occurs with intermittent streaming. On death, the granular structure produced is much less vacuolar.

From the cited experiments, it is observed that the injected hairs survive immersion in a lethal Fe solution averaging 240 per cent. longer than the uninjected hairs. The most favorable experiment at hand using sodium oxalate shows that the average life of 3 injected hairs is 350 per cent. longer than that of the uninjected hairs when immersed in a toxic Fe solution.

TABLE III

RESPONSES OF ROOT HAIRS OF *Trianea* TO INJECTION OF SODIUM OXALATE FOLLOWED BY IMMERSION IN FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	Injected 4 hairs, observed 2
5	Immersed root in Fe solution (10 ppm. of Fe)
7	Many uninjected hairs burst at tip
10	All uninjected hairs burst at tip Injected hairs normal
13	Injected hairs reducing cyclotic rate; protoplasm in one hair very viscous
15	1 injected hair has formed a coagulum, brown, granular, dead
19	Second injected hair has formed coagulum, brown, granular, dead

TANNIN (FIFTH SATURATED SOLUTION).—Injected with this solution, root hairs were immediately irrigated with a solution of ferric nitrate (10 ppm. of Fe). The results are presented in table IV.

TABLE IV

RESPONSES OF ROOT HAIRS OF *Trianea* INJECTED WITH TANNIN SOLUTION, FOLLOWED BY IRRIGATION WITH FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	3 hairs injected, 2 observed
1	Immersed in ferric nitrate (10 ppm. of Fe)
3	Many cells burst
4	All uninjected cells burst, injected cells normal
14	Rate of cyclosis decreasing, protoplasm massing at tip, hair becoming vacuolate
17	Streaming almost imperceptible
20	Coagulum formed in 1 cell, brown, granular; vacuoles disappear to some extent
22	Coagulum formed in second cell, brown, granular; vacuoles smaller and fewer

Tannin is somewhat toxic to these cells when injected. A saturated tannin solution causes the coagulation and death of the cells within a few seconds after injection. Half saturated tannin solutions were also unsatisfactory. However, a fifth saturated tannin solution is apparently harmless to the cells in the quantities injected within the time limits of the experiments.

It is remarked that cells injected with tannin live 650 per cent. longer in a lethal iron solution than uninjected cells. This duration is average for the experiments performed.

HAEMATOXYLIN (SATURATED SOLUTION).—Injected with this solution, root hairs were irrigated with a solution of ferric nitrate (10 ppm. of Fe). The tendency is indicated in table V.

TABLE V

RESPONSES OF ROOT HAIRS OF *Trianea*, INJECTED WITH SATURATED HAEMATOXYLIN, AND IRRIGATED WITH FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	Injected 6 hairs, observed 2
2	Immersed in ferric nitrate (10 ppm. of Fe)
4	All uninjected hairs burst
6	Slight acceleration in streaming in injected hairs
12	Cyclosis normal
15	Decrease in rate of streaming
18	Streaming perceptibly decreases
19	Formation of vacuoles, no cyclosis
20	Coagulum formed in one hair, brown, granular; dead. Decrease in number of vacuoles
24	Cyclosis imperceptible in second hair
26	Brown, granular coagulum formed, dead. Few vacuoles present

In a lethal iron solution, hairs injected with haematoxylin live 1100 per cent. longer than the uninjected hairs.

It is apparent that the injection of haematoxylin, tannin and sodium oxalate into the root hairs of *Trianea* enables those root hairs to survive immersion in toxic and lethal iron solutions significantly longer than uninjected root hairs. Depending on the quantity of injected material in the cells, the time advantage of survival is indefinitely increased.

Discussion

1. The relative non-penetration of Ca.

Small quantities of Fe injected into the cells show a great toxic action. Immersion experiments with this toxic ion exhibit practically the same results with the same material. Immersion experiments with Ca in concentrations up to 0.001 M. show no effect whatever on the protoplasm of the hairs but when small dilute quantities are injected, death results through the formation of a gel, followed by irreversible coagulation. If Ca were as penetrating as Fe, the same results, or at least comparable results, should be obtained on immersion as well as on injection.

2. The antagonism of Ca for Fe.

Immersion experiments (table II) clearly indicate this antagonism. Fe does not enter the cell because Ca is present in the same solution. This action of Ca is strictly a temporary effect for as soon as it is removed, the

characteristic Fe toxicity appears. The root hairs behave precisely the same whether they are *immersed* in an Fe solution or *injected* with same Fe solution.

3. "The external part of the membrane only is affected."

From the above data, this conclusion must follow. Injections are made into the vacuole of the cells. Indeed, it would be almost impossible to inject the cytoplasm. When Fe alone is injected it diffuses rapidly to all parts of the hair, causing simultaneous death of all the protoplasm. Ca remains rather local in its effects, although toxic, forming a viscous gel. The gel with Ca has never been observed in any of our immersion experiments. Therefore no significant quantities of Ca penetrate the cytoplasm beyond the immediate periphery (the plasma membrane) when root hairs are immersed in solutions containing this ion. When the hairs are immersed in solution of both Ca and Fe ions, cyclosis remains normal as does the general appearance of the protoplast. Did Fe penetrate beyond the immediate surface, the effect of its entrance should parallel that seen in injection experiments. Therefore, Ca antagonizes Fe by decreasing the degree of permeability of a definite physiologic, peripheral membrane, the plasma membrane. These observations are in harmony with a great deal of other work (**7, 8, 17, 21**).

These data indicate (but do not demonstrate) that the plasma membrane is composed of a different type of cytoplasm than the vacuolar membrane. Otherwise Ca should remain isolated in the vacuole when injected there and should antagonize Fe by its action on the vacuolar membrane. That this is not the case seems likely.

4. The protective action of substances dissolved in the vacuole.

When cells of great "vitality" or "degree of health" were injected with solutions of tannin, oxalate, or haematoxylin and immersed in toxic Fe solutions, they survived the uninjected cells for hours. Such experiments were impractical and so the time advantage of "sensitive" hairs is recorded. These substances in the concentrations and amounts used are not harmful to the cells. Whether they remain in the vacuole or diffuse out of the cells is conjectural. But so long as they are present, toxic Fe solutions have no effect, although the Fe enters the cell. This latter statement is checked in two ways:—First, the check cells are quickly killed; and second, when the injected substances are precipitated, the injected cells are killed. It is believed, but not demonstrated, that such protecting substances are an ecologic factor in the distribution of plants. The work of Molisch (**15**) is suggestive on this point.

5. The effect of temperature on the rate of permeability.

When *Trianea* is grown below 14° C. for over a week, the cyclotic rate is consistently slower than when the plants are kept at higher temperature. Also the hairs appear to be less permeable to toxic Fe solutions. Material

grown above 23° C. is extremely permeable to the same Fe solutions; and, further, Ca appears powerless to decrease the degree of permeability of the cells.

Conclusions

1. In single salt solution, Fe readily penetrates the root hairs of *Trianea bogatensis.*
2. Ca is a relatively non-penetrating ion.
3. Ca decreases the rate of entry of Fe into the hairs.
4. In the antagonism of Ca for Fe, the "external part of the plasma membrane only is affected."
5. Some evidence is presented suggesting that the plasma membrane is physiologically different from cytoplasm.
6. Such substances as sodium oxalate, tannin, and haematoxylin, when present in the cell, protect the cell from the lethal action of Fe.

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ENERGY EMANATION DURING CELL DIVISION PROCESSES (M-RAYS)

D. N. Borodin

(WITH THREE FIGURES)

Experiments with M-Rays (mitogenetic rays) conducted by the author since September 1928 are partly a continuation of work done by a small group of Russian investigators whose only technical mistake perhaps has been somewhat too soon to call themselves "Practical Vitalists." The problem of the M-Rays (mitogenetic rays) has very little to do with vitalism as a doctrine, but its study has presented some new facts pertaining to the field of biophysical phenomena which occur during cell division processes in general.

Haberlandt's (**10, 11, 12**) theories of cell-division hormones, wound-hormones, necro-hormones, lepto-hormones, etc., affecting cell division and the growth of tissues as a whole, are a purely biochemical explanation of some cellular processes. The physical side of the problem was not at all taken into consideration by Haberlandt.[1]

An explanation on a purely biophysical basis has been presented by Gurwitsch (**4**) in his experiments on the induction of cell division processes in the onion root meristem by exposing it to another onion root. Biophysical induction has been more recently found to occur in plants and protista[2] by Rawin (**16**) in sunflower root tips; Frank and Salkind (**3**) in sunflower seedling leaves; Kisliak-Statkewitsch (**13**) in potato leptom; Wagner (**19**) in *Vicia faba* root-tips; Baron (**1**) in yeast cultures; Magrou and Magrou (**14**) in *Bac. tumefaciens;* Baron (**2**) in *Bac. anthracoides, Sarcina flava, Bac. coli commune,* etc. Most of this work has been done in Russia, but the results have been published in French and German journals.

Owing to the established prestige of Haberlandt, the biophysical explanation of phenomena which he regarded as biochemical, has not gained popularity. The results obtained by Russian colleagues have been ignored in Western Europe for about seven years, until first efforts of verification

[1] Went, (**20**), mentioned that his son found that there is definite emanation from the upper part of a plant (tip) which affects the lower part (stump) by changing the direction of its growth, the two parts being completely separated by the interposition of an artificial culture medium. The explanation suggested was also of a biochemical nature.

It has been noticed also that two pieces of homologous animal tissue planted together but at a short distance from each other (in tissue culture), stimulate the growth of each other. In this case no definite explanation has as yet been offered.

[2] Animals not mentioned.

of the results obtained by them were made by MAGROU and MAGROU (**14**) in Lyon, France. MAGROU repeated the experiments with success and has expressed himself in favor of the presence of inductive forces. Positive results outside of Russia have been also obtained so far by WAGNER (**19**) in Prague; REITER and GABOR (**17**) in Berlin Siemensstadt; and by BORODIN in New York.

ROSSMAN (**18**) in Rostock repeated the original experiments with the onion-root meristem but failed to obtain positive results. This may, however, be ascribed to inadequate technique. His protocols, before publication, were used by his colleague, VON GUTTENBERG (**8**) of Rostock, in the three articles in which VON GUTTENBERG published a very sharp criticism of the theory of M-Rays (mitogenetic rays). VON GUTTENBERG himself never did any of the experimental work in this field.

This short report on experiments with M-Rays prepared for the New York meeting of the American Association for the Advancement of Science in 1928 is confined to plant tissues and, therefore, does not include results obtained by me with animal tissues, nor does it include my studies on the physical and optical properties of the M-radiations.

The technique for the detection of M-Rays (mitogenetic rays) from the onion-root meristem has not yet been described in English. The following is only a brief statement of the technique which will be described in detail in a forthcoming review.

A single onion root connected with its whole bulb, or with a part of its bulb, was placed in a vertical capillary tube of quartz (fig. 1 D) and kept moist. The meristem of this root was used as the "detector" of the radiation. Another onion root, also connected with its bulb (entire or in part), was placed horizontally in a glass capillary tube (fig. 1 S) and also kept moist, and used as the "sender" of radiation. Both tubes are fixed to a special "inductorium," consisting of glass or celluloid plates 7.5 x 5.25 mm. with three more small plates cemented to it in such a way that the distance between the plates is about 1.25 mm. (fig. 1).

In such a position the roots (fixed in the inductorium) were exposed for varying times, from one hour to 2 hours and 30 minutes. The unexposed side of the "detector" was marked with India ink after the induction, and then immediately fixed in Bouin-Allen fluid, etc., for the purpose of sectioning.

In fixed and stained sections the mitoses on the "induced" (exposed) and "noninduced" (nonexposed) sides were counted and the difference in numbers is given in percentages to show the relation between the number of mitoses on the exposed and nonexposed sides. This difference given in percentage we shall call the percentage of induction or, in brief, "Ind. per cent."

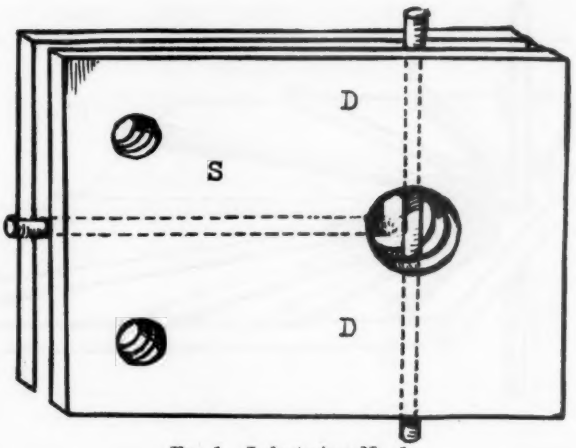

FIG. 1. Inductorium No. 1.

The results of the experiments with onion-root meristem, when the inductoria described above were used, are all positive, as is shown in tables later. It will be seen, however, that the induction percentage varies to a considerable extent. This variation can be explained by the fact that the centering of the "sender" on the "detector" was not fully controlled. In a later experiment the centering was better controlled by using a system of good Leitz mechanical stages together with a horizontal microscope. This equipment was still later replaced by a special inductorium which I have named "condenser." Of this type two modifications were made.

The first modification of inductorium-condenser was made by using an additional capillary glass tube with another onion root as an extra sender. Later, about ten capillary glass tubes (fig. 2) have been used and placed radially on one common glass or celluloid plate of 7.5 x 5.25 mm., pointing them to a common center, which was at a distance of 1 mm. from the end of each tube. In this center the quartz capillary tube with a detector root has been placed vertically, as is shown in fig. 2 D. The results obtained by this type of inductorium were more uniform.

The "condenser" just mentioned has worked quite satisfactorily, but finally was substituted by two object glasses, or celluloid plates, of the same size fastened together. The onion root mash, or other senders, was placed and fixed between these plates as is shown in fig. 3 S. This "sender holder" with some modifications has been quite satisfactory.

In these experiments the onion-root-tip meristem was used, since it is an object which already has been studied very carefully by other investigators.[3]

[3] The average deviation in number of mitoses in opposite sides is estimated as $\pm 8 - 13$ per cent.

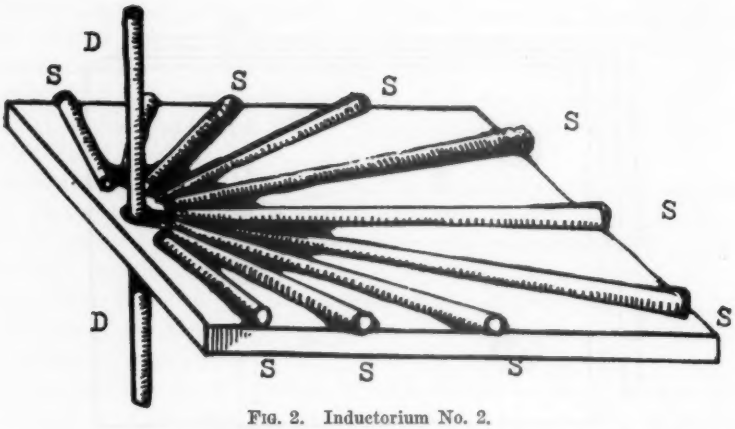

FIG. 2. Inductorium No. 2.

The first experiment was with homoinduction. The "sender" in this set of experiments was a fresh, healthy onion root of one of the three commercial varieties common on the markets of New York: Spanish, Texas red, and Texas white. The "detector" was another onion root of the same variety.

I offer two new signs: ≠, to indicate the "sender;" and ⊙ to indicate the detector.

The purpose of this experiment was, first, to prove the existence of energy emanation from the onion-root tip; second, to prove the existence of an induction at a distance and through media which prevent the possibility

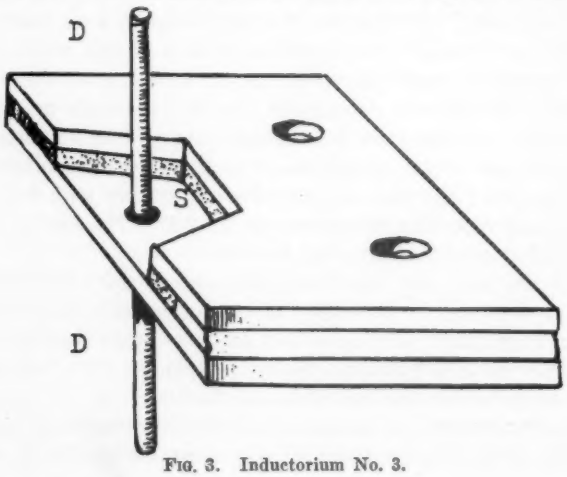

FIG. 3. Inductorium No. 3.

of chemical action; in other words, to prove the physical character of induction.

Homoinduction

The protocols of the experiments with homoinduction are shown below.

Experiment no. 11c

Homoinduction: Sender ≠, onion root tip, variety Texas white. Detector ⊙, same variety, meristem. Apparatus, inductorium no. 1. Separation, quartz capillary tube, and water. Distance, 1 mm. Time of exposure, 2 hours 15 minutes. Fixation, by Flemming. Staining, by iron haematoxylin in sections of 10 μ thickness.

	Mitoses			Resting full-sized nuclei		
(a) Exposed side mitoses, per cent.	31	37	42	41	38	51
(b) Unexposed side mitoses, per cent.	25	30	38	39	27	38
(c) Difference	6	7	4	2	11	13
(d) Induction, per cent.	+24	+23	+11	+5.1	+40	+34

Results: Definite induction of onion-root meristem through quartz.

Experiment no. 12

Homoinduction: Sender ≠, onion root tip, variety Texas white. Detector ⊙, root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz capillary tube and water. Distance, close touch. Time of exposure, 2 hours 30 min. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	56	62	82	60	28	43	55	40	32	18
(b) Unexposed side mitoses	33	35	32	25	20	21	28	30	18	15
(c) Difference	23	27	50	35	8	22	27	10	14	3
(d) Induction, per cent.	+69	+76	+156	+140	+40	+104	+96	+33	+77	+20

Results: Exposure of an onion root meristem, used as detector against the onion root tip of the same variety used as sender at a distance of 1 mm. for 2 hours 30 minutes, through quartz glass results in definite induction or in an increase in the number of ripe nuclei on the exposed side or in comparison to that of the unexposed side. Average, + 81 per cent.

Experiment no. 13

Homoinduction: Sender ≠, onion root tip, variety Texas white. Detector ⊙, root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz plate 2 mm. thick from the side. Distance, 1 mm. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	46	38	58	60	52	57	62	64	52	51
(b) Unexposed side mitoses	30	31	40	45	40	45	42	48	41	48
(c) Difference	16	7	18	15	12	12	20	16	11	3
(d) Induction, per cent.	+53	+20	+44	+33	+30	+26	+47	+33	+38	+6

Results: Same as in experiments nos. 11C, 11D, 12. Average, + 32 per cent.

Experiment no. 14

Homoinduction: Sender ≠, onion root tip, variety Texas white. Detector ⊙, onion-root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz. Time of exposure, 2 hours 30 minutes. Fixation, Gibson. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	62	30	85	63	80	64	59	58	58
(b) Unexposed side mitoses	44	28	39	45	50	45	36	41	62
(c) Difference	18	2	46	18	30	19	23	17	−4
(d) Induction, per cent.	+41	+7	+118	+40	+60	+42	+64	+41	−6

Results: Same as in previous experiment. Average induction, + 45.2 per cent.

Experiment no. 15

Homoinduction: Sender ≠, onion-root tip, variety Texas red. Detector ⊙, onion-root meristem, same variety. Apparatus, inductorium no. 4. Separation, quartz and air. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	72	66	75	80	82	76	61	49	35
(b) Unexposed side mitoses	55	39	45	45	42	44	50	21	20
(c) Difference	17	27	30	35	40	32	11	28	15
(d) Induction, per cent.	+30	+69	+66	+77	+95	+72	+22	+133	+75

Results: Definite induction. Average, + 71 per cent.

Heteroinduction

It has been stated a number of times before that induction is not limited to the action of a species or variety upon itself, but different biological sources may be used as sender for a given detector. That is, induction by one varietal sender and a different varietal detector is possible. In other words, not only homoinduction but heteroinduction can be produced. To prove this, a set of experiments have been conducted with different plants.

Experiment no. 16

Heteroinduction: Sender ≠, hyacinth root, of unidentified commercial variety. Detector ⊙, onion-root meristem, variety Texas red. Apparatus, inductorium no. 1. Separation, quartz capillary tube and water. Time of exposure, 2 hours. Fixation, by Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	32	56	32	69	35	43	38	65
(b) Unexposed side mitoses	26	32	19	42	30	26	34	30
(c) Difference	6	24	13	27	5	17	4	35
(d) Induction, per cent.	+23	+75	+68	+64	+16	+65	+11	+11

Results: Definite induction. Average, + 41 per cent.

Experiment no. 17

Heteroinduction: Sender ≠, willow root, unidentified species from Kew Gardens, Long Island, N. Y. Detector ⊙, onion root meristem, variety Texas white. Both in inductorium no. 1. Separation, quartz capillary tube and air. Distance, 1 mm. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side	51	43	44	63	50	44	65	58	72	52
(b) Unexposed side	35	41	33	44	33	42	31	43	34	44
(c) Difference	16	2	11	19	17	2	34	10	38	8
(d) Induction, per cent.	+45	+4.8	+33	+43	+51	+4	+109	+20	+110	+18

Results: Definite induction. Average, + 43.7 per cent.

In studying the cause of ROSSMAN'S failure (**18**) to obtain positive results I conclude that there were two weak points in his technique: (1) the poor centering of roots during the experiments, and (2) improper fixation solution used for the root tips serving as detectors.

The simplest method of detecting energy emanation from different senders of a biological or physical character is the use of yeast culture on agar blocks. This method excludes the necessity of microtome technique, and was worked out by BARON (**1**). The writer used the micro-incubator placed on the microscope table with quartz object glass, through which induction was made.[4]

The induced yeast culture, after exposure, has been placed on the object glass by means of a platinum loop, fixed and stained. The check control sample from the uninduced (unexposed) culture has been placed by the same method on another object glass, fixed and stained in the same way. The percentage of budding cells has been counted on both object glasses and the difference found. A few figures obtained are shown below.

EXPERIMENT NO. 78

Heteroinduction: Sender ≠, *Bacillus anthracoides*, bouillon culture. Detector ⊙, yeast culture, 12 hours glucose bouillon agar. Apparatus, micro-incubator. Temperature, 32° C. Separation by quartz, 1 mm. thick. Distance, 2 mm. Time of exposure, 2 hours.

(a) Exposed culture budding, per cent.	75
(b) Unexposed culture budding, per cent.	41
(c) Difference	34
(d) Induction, per cent.	+83

Results: Presence of induction shown.

EXPERIMENT NO. 79

Heteroinduction: Sender ≠, *Bacillus anthracoides* culture. Detector ⊙, yeast culture, *S. cerevisiae*. Apparatus, celluloid and quartz plates in a thermostat. Temperature, 32° C. Distance, 2 mm. Time of exposure, 2 hours.

(a) Exposed culture budding, per cent.	40
(b) Unexposed culture budding, per cent.	21
(c) Difference	19
(d) Induction, per cent.	+90

Results: Presence of induction shown.

EXPERIMENT NO. 75A

Heteroinduction: Sender ≠, onion root, variety Texas red. Detector ⊙, yeast culture. Apparatus, micro-incubator. Separation, 1 mm. quartz object glass. Temperature, 32° C. (89° F.). Distance, 3 mm. Time of exposure, 2 hours 30 min.

[4] Later on, a special inductorium was constructed, consisting of a quartz plate, diaphragm, heating element, and a glass cylinder for yeast culture. Optimum temperature was controlled by a rheostat.

(a) Exposed culture budding, per cent.	65
(b) Unexposed side budding, per cent.	41
(c) Difference	24
(d) Induction, per cent.	+59

Results: Definite induction shown.

My experiments confirm the existence of energy emanation during the cell division processes in four biological objects: onion root, hyacinth root, willow root, and bacteria. The energy is in the form of radiation, penetrating through quartz glass, and stimulating the tempo of the cell division process of the onion-root tissues and yeast cell colony. Tissues of different plants, yeast or bacteria cells can affect each other as well. The effect is noted at a distance.

The dividing cell has proven to be as good a detector of energy as the most sensitive physical instruments, and may perhaps be even more sensitive. The sensitiveness of a living organism and its use as a most delicate instrument recalls the experiments of PACKARD (**15**) with *Drosophila* eggs, used for quantitative determination of Roentgen units.

The existence of a specific energy emanation in a living cell may be of greater significance than it appears at the present, and should be studied carefully. Experiments thus far conducted show definitely that it has a stimulating, if not a specific, effect on the living cell or at least on its nuclei to the extent of inducing their division directly or indirectly. The theory of M-Rays (mitogenetic rays) as a working hypothesis will stimulate research in the field of experimental cytology, general physiology, as well as in the field of genetics, particularly in the study of gene activity. At any rate, the investigation of the physical side of the problem will thus be promoted.

The organism as a whole has the same functional elements as a cell has in miniature. The organism is a field of activity, as it were, of a tornado of chemical and physical forces. Whereas the chemical side has been studied carefully and some questions already have been answered, the physical side of the processes in the living organism has not yet been an object of intensive study, owing to the fact that scientists did not possess the delicate instruments which are now coming into use by modern physicists: thermocouples, photoelectric cells, hydrogen-ion concentration apparatus, etc.

We know at present how powerful an influence X-Rays, radium, and even temperature, phenomena of a physical category, have on the living organism. These forces, in the hands of a scientist, have already been shown to produce considerable changes in the most hidden parts of an organism, *viz.*, the germ-plasm. All these forces are produced artificially and

outside the organism. We know very little, however, about the existence of these forces in the living organism, and they are wondered at when they are occasionally discovered. Can an organism be a producer of heat, light, or electricity? The answer is—yes, for it has been readily detected. But can the organism be a source of ultra-violet rays, X-Rays, or other "unusual" radiations? We are still in doubt about this. Many of us still answer—"impossible."

From the studies of M-radiation we know now that the *cells* possess the ability of some energy emanation which has been likened to ultra-violet radiation of a definite wave length. The source of this energy emanation has been shown to be the property of living cells the activity of which stimulates some of the life processes.[5]

There are many possibilities that this theory will have a far-reaching application in the field of genetics. It is of interest to note that of the 62 papers presented at the section of genetics at the New York meeting of the A. A. A. S. in 1928, 12 were devoted to the action of X-rays.[6]

The induction by M-Rays (mitogenetic rays), biophysical forces, at a distance, effects as far as we know at present, the division of nuclei and of chromosomes *in toto*. The possibility of a selective effect on parts of chromosomes is not excluded. The X-ray effect, if not selective, may at any rate, according to one theory, destroy a complete molecular complex comprising a gene, thereby changing the balance. The result is the dislocation of other molecular parts in the field created by X-rays, and destruction of this group. According to another theory, X-rays rearrange the molecular structure of a part of a chromosome or of a gene.

In all experiments with the breeding of *Drosophila* under varying temperatures, from 19° C. to 30° C., commercial yeast cultures belonging

[5] We can now almost suspect the close connection between the origin of vitamins and activity of this radiation. Both vitamins and radiation are essential in substances used by organisms as food. Vitamin D, as antirachitic, has been shown to depend somewhat on ultra-violet radiation. Here is a wide horizon for fruitful research and explanations.

[6] The molecular components of chromosomes, chromomeres, chromioles and even genes may be considered as the specific structural centers of organic function. We may assume that each molecular unit is produced from similar molecular units with no change in the chemical composition and with no changes in the physical properties. The chemical homogeneity of the molecular units of the chromosome parts is the basic foundation of heredity. The production of more molecular units of the same character is only possible by longitudinal division of the chromosome (*"omnis molecula ex molecula"*). This comprises the equal division of every molecular component. Thus it repeats all the complex characteristics of a single gene complex comprising a chromosome. The biochemical and bio-physical activity of a single group of molecular units, not more than the size of octokaidekapeptid, or about 0.01 μ, in bunches close together, may be identified with the activity of a gene which is conceived to be a complicated center of combined activity of a single group of molecular units.

to the strain of *Saccharomyces cerevisiae* were used as food. According to some statements, the maximum multiplication and maximum number of mutations occur at a temperature close to 30° C. I should like to point out the fact that the optimum of multiplication of yeast cultures of this particular race occur at this same temperature or about 28°–29° C.

Is the change in temperature itself the *only cause* of the increase of multiplication and mutations of *Drosophila?* Or is it the result of radiation emanating from the yeast as well? We have no proof to confirm this, but it would be of interest to use different species of yeasts whose optimum multiplication occurs at different temperatures from the above (below 19° or above 29° C.). A study of the progeny of *Drosophila* and of the number of mutations occurring under these new conditions may show some interesting results.

The source of M-Rays (mitogenetic rays) in a living cell has not been definitely located, but it has been found already that not all the tissues of plants and animals possess this peculiarity in equal measure. The possibilities of the location of this activity in the nuclei or chromosome substances is not to be excluded. If it should prove to be the case that the radiations arise from the nuclei or chromosomes, a new page in the history of the study of gene activities will be opened. At present, however, we only know that there is a definite connection between the intensity of the metabolic processes and the emanation of M-rays.

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TOXICITY OF SOME ALIPHATIC ALCOHOLS

WALTER S. EISENMENGER

(WITH SIX FIGURES)

Previous investigations have revealed the importance of certain ions for plant life, and the use of mixtures of salts in aqueous solution containing these ions has led to a conception of a favorable medium for complete plant development.

Nutritive value of solutions

The researches of KNOP (**8, 9**) and BIRNER and LUCANUS (**1**) showed that higher plants require nitrogen, sulphur, phosphorus, calcium, magnesium, iron and potassium ions in an aqueous solution about their roots.

More recently MCHARGUE (**19**) has shown that in the total absence of manganese plants do not develop normally. JOHNSTON and DORE (**7**), SOMMER and LIPMAN (**31**), SOMMER (**30**), and others have shown that boron is essential. Additional evidence [SOMMER and LIPMAN (**31**)] has indicated that zinc is needed.

The first investigation that led to a systematic testing of logically complete series of salt proportions was by TOTTINGHAM (**32**). This work, in addition to that of SHIVE (**29**) and others, has indicated that best growth can be secured with a solution having an osmotic value of 0.25 to 2.50 atmospheres. Very concentrated solutions may exert a physical influence in retarding the absorption of water by the plants, as well as a toxic effect, if their components are not properly balanced; extremely dilute solutions though practically non-toxic may fail to supply the essential chemicals rapidly enough for best growth.

Several improvements in solution-culture technique have been given attention by TRELEASE and FREE (**33**), and TRELEASE and LIVINGSTON (**34**); of selecting seeds for uniformity of weight by TRELEASE and TRELEASE (**35**).

LOEB (**12, 13, 14, 15**) was the first to describe the antagonistic action of salt solutions. He was the first to discuss clearly the difference between balanced solutions and nutrient solutions. LOEB discovered that young fish (*Fundulus*) soon die if placed in a solution of NaCl of the concentration in which this salt is contained in sea water. When KCl and $CaCl_2$ were added to the solution in the right proportions the fish could live indefinitely.

Antagonism was considered by LOEB to depend upon an action of both salts and the egg membrane, whereby the membrane becomes nearly impermeable to both salts. The toxicity of each salt in simple solution was assumed to be due to its diffusion through the membrane.

OSTERHOUT (**21**) emphasizes the fact that the injurious effects of single salt solutions cannot be due to the lack of nutrients, for distilled water is less harmful than simple solutions. OSTERHOUT (**22, 23, 24**) calls attention to the fact that in order to know the degree of antagonism, it is necessary to know the additive effect—the total toxic effect that the solution would exert if no antagonism existed and each simple solution exerted its toxic effect independently. By comparing the additive effect with the effect in a mixed solution it can be seen whether the toxicity of the two salts is unaltered (the same as the additive effect), whether the toxicity is diminished (less than the additive effect), or whether the toxicity is increased (greater than the additive effect).

With respect to the toxicity of the simple monohydroxy alcohols little of a quantitative nature has been reported. RICHARDSON (**27, 28**) states, regarding their toxicity, that they change in character regularly as the carbon chain increases in length. He found the death point for *Medusa* in ethyl alcohol to be at a concentration of from 0.03 to 0.1 per cent.

HODGE (**6**) found that when ethyl alcohol is present in yeast cultures to the extent of 0.01 per cent., 992 cells of yeast were produced instead of 2061 under normal conditions. In this instance there was retardation of cell division due to the presence of alcohol.

RAUBER (**26**) used ethyl alcohol and found that roots of *Impatiens* were killed in a 5 per cent. ethyl alcohol solution. In a 20 per cent. solution *Pinus larix* turned yellow, and a 5 per cent. solution destroyed fungi when applied to soil around them.

MALTAUX and MASSART (**17**) reported that in the case of *Paramecium* there was an increase in cell division when treated with dilute ethyl alcoholic solutions. This characteristic was later lost.

Alcohols have been regarded as protoplasmic poisons. However, DANIEL (**3**), who used two strains of *Stentor*, reports that these organisms can acclimate themselves to a degree in very dilute solutions of ethyl alcohol. In a 1 per cent. solution they lived as long as one week in a favorable condition. A 2 to 3 per cent. solution produced death after a period of six and two hours respectively. In a 4 per cent. solution they always died. They were not resistant to an 8 per cent. solution but died in eighty to one hundred seconds. In a 1 per cent. solution of methyl alcohol *Stentor* became acclimated to the medium. From the data cited, it seems that low concentrations of alcohol in these instances constituted a lethal dose for both plants and lower animals.

For higher forms of animal life, MUNCH and SCHWARTZE (**20**) found the average lethal dose for rabbits to be as follows: Cubic centimeters of alcohol per kilo weight: Methyl 18, ethyl 12.5, normal propyl 3.5, isopropyl

10, normal butyl 4.25, isobutyl 3.75, secondary butyl 6, tertiary butyl 4.5, isoamyl 4.25, secondary amyl 3.5, tertiary amyl 2.5. In this instance the order of relative toxicity is to a large degree as it is in plant life.

Criteria of growth

In this investigation two criteria of growth were employed: (1) elongation of roots of germinating soy-beans; (2) the ash of the shoot (stem and leaf). The initial behavior of germinating beans involves less complexity within as well as without the organism than is involved in later phases of growth. In the initial stages of growth the plants are well supplied with organic food from the seed, and the supply of salts is sufficient to afford protection from a condition of starvation of roots grown in distilled water. The protoplasm of the roots appears to be readily accessible to the toxic agent.

The three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, were chosen for the preparation of the control solution because of their importance in supplying ions needed in normal growth in plants.

In an extended investigation SHIVE (**29**) found that wheat plants supplied with these three salts grew during the first four weeks after germination as well as they did in solutions containing a greater number of salts.

In this investigation a study was made of the toxicity of the following alcohols: methyl, ethyl, normal propyl, isopropyl, normal butyl, isobutyl, secondary butyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl, and also that of calcium nitrate. The concentration of each alcohol or salt ranged from 0.0012 M. to 0.06 M. (2, 5, 15, 30, 50, 85, 98, and 100 per cent. of 0.06 M.). Tests to show possible antagonism were made by using mixed solutions, each containing a normal alcohol and calcium nitrate—namely, methyl + $Ca(NO_3)_2$, ethyl + $Ca(NO_3)_2$, normal propyl + $Ca(NO_3)_2$, normal butyl + $Ca(NO_3)_2$, normal amyl + $Ca(NO_3)_2$. With each concentration, ten sets of molecular proportions of alcohol and calcium nitrate were used as follows: 0 + 100, 2 + 98, 5 + 95, 15 + 85, 30 + 70, 50 + 50, 70 + 30, 85 + 15, 98 + 2, 100 + 0.

Methods

The methods employed were essentially the same as those described by TRELEASE and TRELEASE (**36**), and by EISENMENGER (**4**). A pure line of soy-beans (Virginia) was used, secured through the courtesy of Professor J. E. METZGER, of the University of Maryland. The seeds were germinated on moist filter-paper in glass culture dishes in a dark room.

For each culture two Pyrex beakers (tall form, without spout) were used. The smaller beaker was of 300-cc. capacity, the larger of 600-cc. capacity. Over the top of the small beaker was stretched a piece of paraffined mosquito netting, which was secured below the rim by a ligature of

paraffined linen thread. The smaller beaker was placed inside the larger one, and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the average length of the primary roots of the seedlings was about 8 mm. selected seedlings were placed on the mosquito netting so that the roots dipped into the culture solution. Duplicate cultures, each of twenty-five plants, were used for each experimental solution. The cultures were placed in a dark room, and the seedlings were allowed to grow until the primary roots of the control culture had acquired a length of about 95 mm. or until these roots had elongated 87 mm. (95 mm. minus 8 mm).

The length of the primary root of each individual plant was then recorded and the average computed. From this average was deducted the average length of the roots at the time when the seedlings were taken from the germination chamber.

This difference constituted the average root elongation value for the culture. The growth data presented in this paper are relative values. Each relative growth value was obtained by dividing the average elongation of a given culture by the average elongation of the control culture and multiplying this quotient by one hundred.

Another criterion of growth was the relative ash content of the shoots of the seedlings. The shoots were cut from the seed when the roots had made new growth of about 87 mm. (95 mm. minus 8 mm.). The shoots were then placed in weighed porcelain crucibles and burned completely to ash in an electric furnace and weighed on an analytical balance. Here again, as in the root elongation data presented, values are relative. Each relative growth value was obtained by dividing the average ash weight of a given culture by the average ash weight of the control culture and multiplying this quotient by one hundred.

The average temperature during the growth of the seedlings was 21° C. The average time required for the roots of the control solutions to acquire an additional length of 87 mm. was 98 hours. Four control cultures of 25 plants each were used. Also, two distilled water cultures of 25 plants each were included in each series. The composition of the control solutions was as follows: 0.02 M. KH_2PO_4, + 0.02 M. $Ca(NO_3)_2$, and 0.02 M. $MgSO_4$.

The water used was obtained from a Barnstead still. The salts were "Baker's analyzed," and the alcohols were Eastman's. The latter conformed in boiling point to the standards usually ascribed to them.

Results of tests with single alcoholic solutions and single salt solutions of calcium nitrate

The relation between root elongation rates and volume-molecular concentrations of single normal alcohol solutions and of single solutions of calcium nitrate are shown in the graphs of figure 1.

The abscissae represent volume-molecular concentrations, and the ordinates represent growth rates expressed as percentages of the root elongation in the three-salt standard or control solution. In each case all of the observed points are shown, and the curve represents what appears to be the general trend of the points. It will be observed that the seven graphs differ greatly in form.

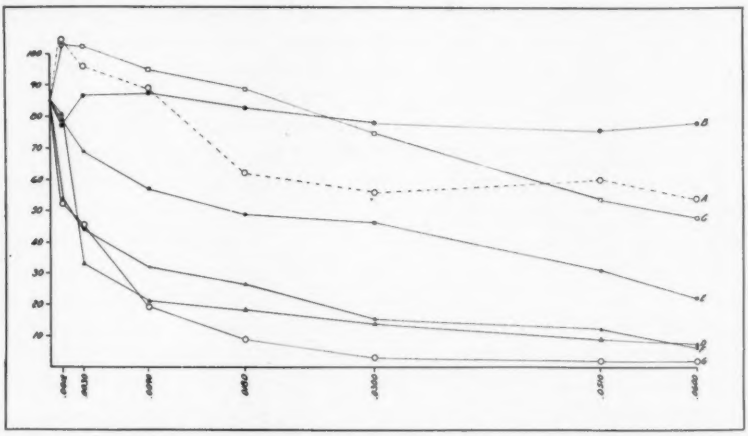

FIG. 1. Elongation of soy-bean roots in simple solutions of normal alcohols, and calcium nitrate. Ordinates represent percentage of elongation for standard solutions; abscissas represent volume-molecular concentrations. A. Calcium nitrate; B. Methyl; C. Ethyl; D. N propyl; E. N butyl; F. N amyl; G. N hexyl.

SOLUTIONS OF CALCIUM NITRATE

The graph for $Ca(NO_3)_2$ in figure 1 rises from 0 molar to 0.0012 M. From 0.0012 to 0.03 M. there is a decided slope downward, and again a slight rise at 0.051 M. The unexpected growth at 0.0012 M. seems to indicate that for soy-beans in their early stages of growth, $Ca(NO_3)_2$ at very low concentrations has a stimulating influence. Wheat seedlings do not show this increased growth effect at low concentrations (**4**).

SOLUTIONS OF METHYL ALCOHOL

Solutions of this alcohol are slightly more toxic than distilled water. At the lowest concentrations it is slightly less toxic than distilled water and at higher concentrations, more toxic than distilled water.

SOLUTIONS OF ETHYL ALCOHOL

Ethyl alcohol in its physiological action differs from other alcohols in that at very low concentrations it exerts a decided stimulating influence. It

allows a growth rate at 0.0012 M. greater than does a standard solution. As the concentration increases from 0.0012 M., there is a gradual increase in toxicity until it reaches the maximum strength used, 0.06 M.

Solutions of normal propyl alcohol

Normal propyl alcohol seems to be one of the most toxic alcohols. A solution of 0.003 M. concentration allows root elongation equal to 33 per cent. of the growth rate in a standard solution. The toxicity gradually increases with concentration until, at 0.06 M., the growth is equivalent to only 7 per cent. of the growth attained in a standard solution.

Solutions of normal butyl alcohol

N-butyl alcohol is more toxic than methyl or ethyl alcohol throughout all concentrations, and less toxic than is N-propyl alcohol. It increases in toxicity with increased concentration.

Solutions of normal amyl alcohol

N-amyl alcohol is approximately as toxic as N-propyl alcohol. At concentration 0.0012 M. it is slightly more toxic, and at concentrations 0.003 M. to 0.06 M. it is slightly less toxic than normal propyl alcohol at the same molar concentration.

Solutions of hexyl alcohol

Throughout all concentrations used except one (0.003 M.) this is the most toxic alcohol. At this concentration, propyl alcohol is slightly more toxic. This difference is so small, however, as to permit the statement that hexyl alcohol is the most toxic alcohol used. The roots in solutions of 0.03 M. to 0.06 M. seemed to be killed soon after being immersed in the solution.

Solutions of isopropyl alcohol

There is little difference in the growth-retarding influence of isopropyl alcohol and methyl alcohol. From concentrations 0.0012 M. to 0.0018 M. this alcohol seems to be approximately as toxic as distilled water. At concentrations higher than 0.0018 M. it is slightly more toxic than distilled water. It is one of the least toxic alcohols.

Comparisons of growth in different alcohols

It is interesting to institute comparisons between the physiological action of some of the alcohols that are similar in structure. Thus, comparing two alcohols of the same number of carbon atoms, propyl and isopropyl, it will be observed that they represent the extremes in their physiological action—the former exceedingly toxic, the latter relatively inert.

In a general way one might classify methyl alcohol and ethyl alcohol together in a group whose physiological action is somewhat alike. At very low concentrations they seem to have a stimulating effect, and at higher concentrations they are less toxic than are the other alcohols. In a group showing high toxicity may be included normal propyl, normal amyl, and normal hexyl alcohol. The general outline of the growth curves seems to indicate that these produce similar toxicity, though not all to the same high degree. Normal butyl alcohol stands midway between these two classes in that it is moderately toxic.

Butyl alcohols

It has been stated by McCollum (**18**) that the toxicity of the alcohols increases with the increasing number of carbon atoms in the molecule, and that the normal alcohols are more toxic than those alcohols in which the atoms are not represented in a straight chain in the molecule.

In fig. 3-B are presented the root elongation curves of the soy-bean in four butyl alcohols. At a low concentration, as 0.003 M., the root elongation in solutions of normal, secondary, and tertiary butyl alcohols is about the same. From 0.003 M. to 0.06 M. there is a decided increase in toxic effects of all three alcohols. This increase is most rapid in the case of normal butyl alcohol, less so in solutions of tertiary butyl alcohol, and least so in the case of the secondary butyl alcohol. Isobutyl alcohol throughout most of the different concentrations exerts the greatest toxic effect of all the butyl alcohols. However, at the low concentration of 0.0012 M. this seemingly toxic alcohol has a stimulating effect. This is of interest, since it illustrates the principle frequently stated in physiological literature, that dilute solutions of a substance which is toxic in higher concentrations may be expected to have stimulating effects.

Amyl alcohol

Among the alcohols thus far discussed no two alcohols containing the same number of carbon atoms per molecule resemble each other more closely with respect to their influence on soy-bean seedling growth than do the two amyl alcohols—normal and isoamyl (fig. 3-A). At low concentrations the normal alcohol is somewhat more toxic than is isoamyl alcohol.

General order of growth effects

At the maximum concentration, 0.06 M., the order of toxicity from least to most toxic is as follows: methyl, isopropyl, secondary butyl, tertiary butyl, ethyl, normal butyl, isobutyl, isoamyl, normal propyl, normal amyl, normal hexyl.

At a concentration of 0.03 M. the order of increasing toxicity is as follows: isopropyl, methyl, ethyl, secondary butyl, tertiary butyl, normal butyl, isobutyl, isoamyl, normal amyl, normal propyl, normal hexyl.

TABLE I

GROWTH IN SOLUTIONS OF ALCOHOLS AND IN CALCIUM NITRATE AS INDICATED BY RELATIVE ROOT ELONGATION

MOLECULAR	AVERAGE OF INDIVIDUAL TESTS OF 50 SEEDLINGS EACH											
	METHYL	ETHYL	NORMAL PROPYL	ISO-PROPYL	NORMAL BUTYL	ISO-BUTYL	SECOND-ARY BUTYL	TERTIARY BUTYL	NORMAL AMYL	ISOAMYL	NORMAL HEXYL	CALCIUM NITRATE
0.0012	78	104	81	81	79	98	87	79	54	71	54	104
0.0030	74	103	33	86	69	83	80	72	44	63	45	96
0.0090	88	95	21	84	57	42	80	71	32	53	20	88
0.0180	83	89	18	86	49	30	69	60	26	30	9	62
0.0300	78	75	14	81	46	27	69	53	15	18	3	56
0.0510	76	54	9	72	31	20	61	50	12	11	2	60
0.0600	78	48	7	75	22	20	59	54	6	9	2	54

The variation from minimum to maximum root elongation is greatest in the case of isobutyl alcohol, ranging from 20 per cent. (of the root elongation in a control solution) at a concentration of 0.06 M. to 98 per cent. at a concentration of 0.0012 M. In methyl alcohol this variation is least, ranging only from 76 per cent. at 0.051 M. concentration to 88 per cent. at 0.009 M. Thus the seedlings grown in solutions of methyl alcohol are least affected by variation of molar concentration, and seedlings grown in solutions of isobutyl alcohol are most affected by variations of concentration. The growth data upon which the foregoing discussion is based are presented in table I.

Concentration and toxic action

An examination of the literature shows that a relatively simple relation between concentration and toxic action has been derived from some studies. Thus CHICK (**2**), working on the effect of disinfectants on the period of bacterial survival was able to state the relation in simple mathematical terms. The process of disinfection was found to obey the laws of a monomolecular reaction, characterized by a definite velocity constant; and a study of the velocity constants for a wide range of concentrations of the disinfectants showed that the velocity of disinfection is a power function of the concentration.

Similar relations have been reported by KRONIG and PAUL (**10**), PAUL, BERNSTEIN and REUSS (**25**), and FALK and WINSLOW (**5**). The same idea is involved in the conclusion of LERENARD (**11**), who reported that the degree of toxicity of any solution may be calculated if we know the coefficient of toxicity of the salt in question and its concentration in solution. Similarly, OSTERHOUT (**23**) states that in most cases equally toxic solutions, if both solutions are diluted to the same degree, will remain equally toxic. It should be observed, however, that the results secured in the present study show that the order of toxicity of several alcohols is not always constant, but varies with the concentrations used.

In the case of the alcohols and calcium nitrate, a mathematical expression has been derived from the data for each compound, which may be used to calculate the approximate relative growth at any concentration falling within the limits of those used—0.0012 M. to 0.06 M. Thus in the exponential approximation by method of least squares the following items are indicated; let a = average (of relative root length or average of relative ash content); m = the molar concentration; e = Napierian base.

For example, we may consider secondary butyl alcohol at a concentration of 0.03 M. The expression for this compound when root length is considered, is: $a = 82.8e^{-6m}$. Now by the theory of logarithms, log a = log 82.8e – 6m log e, but in the Napierian system log e equals unity. Therefore

log a = log 82.8 − 6m or log a = log 82.8 − 6 (0.03) and by simplifying, log a = 4.416 − 0.18 or 4.236. The number corresponding to this natural logarithm is 69. In this instance the calculated value is identical to that representing the relative root length at the concentration of 0.03 M.

The following are the values thus calculated:

	For root length	For ash content
1 Methyl	$a = 80.3e^{-.5m}$	$a = 48.9e^{-1.2m}$
2 Ethyl	$a = 108.5e^{-13.4m}$	$a = 70.7e^{-12.5m}$
3. Normal propyl	$a = 41.4e^{-31.6m}$	$a = 36.7e^{-21.2m}$
4. Isopropyl	$a = 85.5e^{-2.5m}$	$a = 57.6e^{-2m}$
5. Normal butyl	$a = 74.1e^{-18.8m}$	$a = 51.6e^{-24.9m}$
6. Isobutyl	$a = 69.1e^{-24.5m}$	$a = 47e^{-16.7m}$
7. Secondary butyl	$a = 82.8e^{-6m}$	$a = 50.5e^{1.1m}$
8. Tertiary butyl	$a = 73.1e^{-6.7m}$	$a = 50e^{0.3m}$
9. Normal amyl	$a = 47.6e^{-32.5m}$	$a = 45.3e^{-34.4m}$
10. Isoamyl	$a = 66.7e^{-35.7m}$	$a = 70e^{-36.6m}$
11. Normal hexyl	$a = 37.4e^{-57.3m}$	$a = 30.3e^{-62m}$
12. Calcium nitrate	$a = 92.2e^{-10.2m}$	$a = 77.6e^{-5.3m}$

Most of the values calculated compare favorably with the actual comparative measure of growth. However, in the case of the most toxic compounds, as hexyl alcohol, and the least toxic compounds, as methyl alcohol and isopropyl alcohol, calculations may be considered as less applicable. The curves representing the toxicity of these compounds tend to flatten out at the low concentrations for the less toxic compounds, or at the higher concentrations for the more toxic compounds.

Comparison of molecular concentrations producing equal growth rates

If any pair of curves discussed had the same slope, it would signify the existence of a constant ratio between the molecular concentrations of two solutions producing equal growth rates. Since the slopes are different, it is of interest to see to what extent the ratios differ, as indicated by table II.

Physical constants as related to toxicity.—The results of these experiments did not seem to justify any statements regarding a relationship between such physical constants as density, specific heat, refractive index, molecular refraction, viscosity, and the toxicity of the alcohols used.

Relation of toxicity to number of carbon atoms in the molecule of alcohol.—Only by making an exception of normal propyl alcohol can it be stated that the toxicity of normal alcohols increases with increase of molecular weight. Incidentally, normal propyl alcohol is the only alcohol used, which is miscible in all proportions with both oil and water.

TABLE II

MOLECULAR CONCENTRATIONS OF SINGLE ALCOHOL SOLUTIONS REQUIRED TO PRODUCE VARIOUS EQUAL ROOT ELONGATIONS

RELATIVE GROWTH RATE	MOLECULAR CONCENTRATIONS OF SINGLE ALCOHOL SOLUTIONS										
	METHYL	ETHYL	N PROPYL	ISOPROPYL	N BUTYL	ISOBUTYL	SECONDARY BUTYL	TERTIARY BUTYL	N AMYL	ISOAMYL	N HEXYL
80	0.0258	0.0258	0.0012	0.0318	0.0012	0.0036	0.003	0.0012		0.0006	
70		0.0354	0.0018		0.0030	0.0048	0.0174	0.0096	0.0006	0.0012	0.0006
60		0.0450	0.0018		0.0078	0.0066	0.0552	0.018	0.0012	0.0048	0.0012
50		0.057	0.0024		0.0174	0.0078		0.051	0.0018	0.0102	0.0024
40			0.0030		0.0390	0.0108			0.0048	0.0144	0.0042
30			0.0048		0.0522	0.018			0.0120	0.018	0.0066
20			0.0120			0.051			0.0246	0.0282	0.009
10			0.048						0.0540	0.0552	0.0174

Ash of shoots

The method of weighing the ash of the stems was adopted to furnish another criterion for determining growth rates. It was not anticipated that the growth rate as judged by root elongation would correspond precisely with the growth rate as measured by the ash content of the stems of the same plant. But as the alcohols themselves furnish no ash constituents to the plant, this criterion of growth would, in a degree, give one an idea as to how much of the inorganic constituents were furnished by the seeds to the stem during a short growing period.

It was found by comparison that the more toxic alcohols inhibit the transportation of plant nutrients from the seed to the stem, and that the ash content of the shoots of seedlings was a fair measure of growth.

The average elongation of seedlings grown in distilled water was 85 per cent. of the average elongation of seedlings grown in a control solution, and the average ash content of shoots grown in distilled water was 52 per cent. of the ash content of shoots grown in a control solution. These data alone reveal the fact that the graphs representing root elongation and the graphs representing ash content of shoots cannot run parallel.

Figure 2 represents the relation between growth rates (as measured by ash content) and volume-molecular concentration of six normal alcohols, and of a single salt solution of calcium nitrate. The order of toxicity of the different alcohols throughout most of the concentrations is practically the same as the order illustrated in fig. 1, where root elongation is plotted against volume-molecular concentration. One of these exceptions is the case of normal propyl alcohol, which by ash content measurement seems slightly less toxic than amyl alcohol at high concentrations. In the case of the root-elongation measurements the former is seen to be the more toxic of the two.

Even more pronounced is the difference in the configuration of the ash and elongation curves for $Ca(NO_3)_2$ (figs. 1 and 2). In the case of ash measurement the curve falls far more slowly than does the root elongation curve (fig. 2), where volume-molecular concentration is plotted against root elongation. In the former figure, the maximum concentration of $Ca(NO_3)_2$ does not appear to exert as much toxic influence as does distilled water. It seems that solutions of salts like $Ca(NO_3)_2$ must contribute directly to the ash content of the seedlings in early periods of growth. However, it is evident that in solutions of high concentration of $Ca(NO_3)_2$, relatively less ash is transported to the seedlings than in cases where smaller amounts of the salt are present.

When the seedlings are grown in various $Ca(NO_3)_2$ solutions, the measure of growth, as indicated by root elongation, does not seem to bear a favorable resemblance to the growth as indicated by the relative ash content.

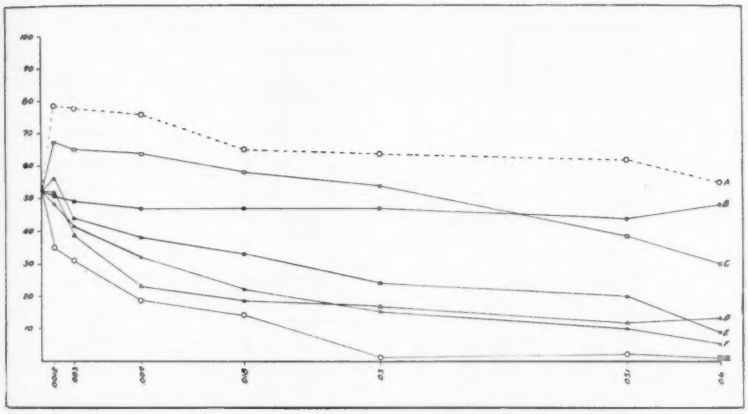

FIG. 2. Ash of soy-bean shoots grown in simple solutions of normal alcohols and calcium nitrate. Ordinates represent percentages of ash for standard solution; abscissas represent volume-molecular concentrations. A. Calcium nitrate; B. Methyl; C. Ethyl; D. Ethyl; E. N butyl; F. N amyl; G. N hexyl.

SOLUTIONS OF PROPYL ALCOHOLS

The relation of growth rate (as measured by relative ash content) and volume-molecular concentration of single solutions of normal propyl and isopropyl alcohol are shown in the graphs of fig. 4-C. The general directional outline of each curve is not greatly different from that where the relation of root elongation to volume-molecular concentration is represented (fig. 3-C). However, as the initial point (distilled water) in each graph is lower than the initial point where the relationship of root elongation to volume-molecular concentration is shown, the curves representing measurement with respect to ash content are not as far apart.

SOLUTIONS OF BUTYL ALCOHOLS

In comparing the toxicity of the different butyl alcohols it is found that the order of increasing toxic properties is as follows: secondary butyl alcohol, tertiary butyl alcohol, normal and isobutyl alcohol. The last two named have approximately the same degree of toxicity. In a general way it may be stated that the butyl alcohols as a class are of moderate toxicity as compared with the slightly toxic alcohols, methyl, ethyl, and isopropyl, and the extremely toxic alcohols, normal propyl, normal amyl, isoamyl, and normal hexyl (fig. 4-B).

SOLUTIONS OF AMYL ALCOHOLS

In fig. 4-A the relationship between gram-molecular volume and ash content of seedlings grown in the two different alcohols, amyl and isoamyl,

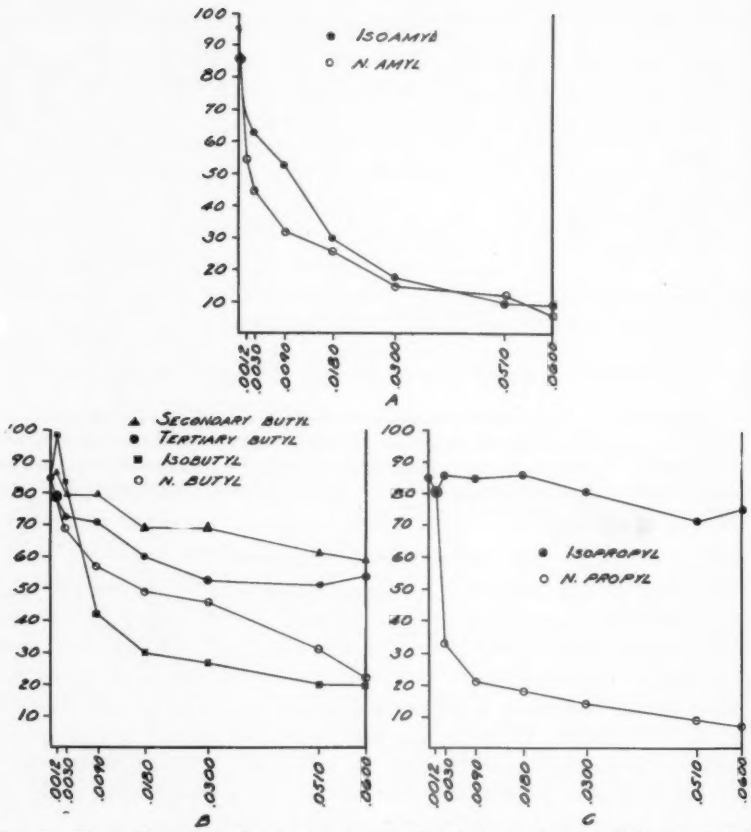

FIG. 3. Elongation of soy-bean roots in simple solutions of eight different alcohols. Ordinates represent percentages of elongation for standard solution; abscissas represent volume-molecular concentrations.

is indicated. Again the normal alcohol is the more toxic, as in the case of the propyl alcohols. However, the difference in the case of the amyl alcohols is not so pronounced. At high concentrations there is little difference in toxicity. There is a marked similarity in outlines of curves as illustrated by figs. 3-A and 4-A. The data as to relative ash content of shoots grown in these alcohols are shown in table III, and the molecular concentrations of alcohol solutions required to produce equal quantities of ash in shoots is shown in table IV.

TABLE III

GROWTH IN SOLUTIONS OF ALCOHOLS AND IN CALCIUM NITRATE AS INDICATED BY RELATIVE ASH CONTENT OF SHOOTS

MOLECULAR CONCENTRATION	AVERAGE OF INDIVIDUAL TESTS OF 50 SEEDLINGS EACH											
	METHYL	ETHYL	N PROPYL	ISO-PROPYL	N BUTYL	ISOBUTYL	SECOND-ARY BUTYL	TERTIARY BUTYL	N AMYL	ISOAMYL	N HEXYL	CALCIUM NITRATE
0.0012	51	67	52	53	56	53	53	53	48	63	35	79
0.0030	49	65	39	57	44	50	47	47	41	60	31	78
0.0090	47	64	23	56	38	39	53	51	32	53	19	76
0.0180	47	58	19	58	33	31	47	50	22	35	14	65
0.0300	47	54	17	52	24	21	56	50	15	26	1	64
0.0510	44	38	12	52	20	22	57	50	10	12	2	62
0.0600	47, 50	37, 24	12, 14	56	11, 7	19	50	52	6, 4	7	1	56

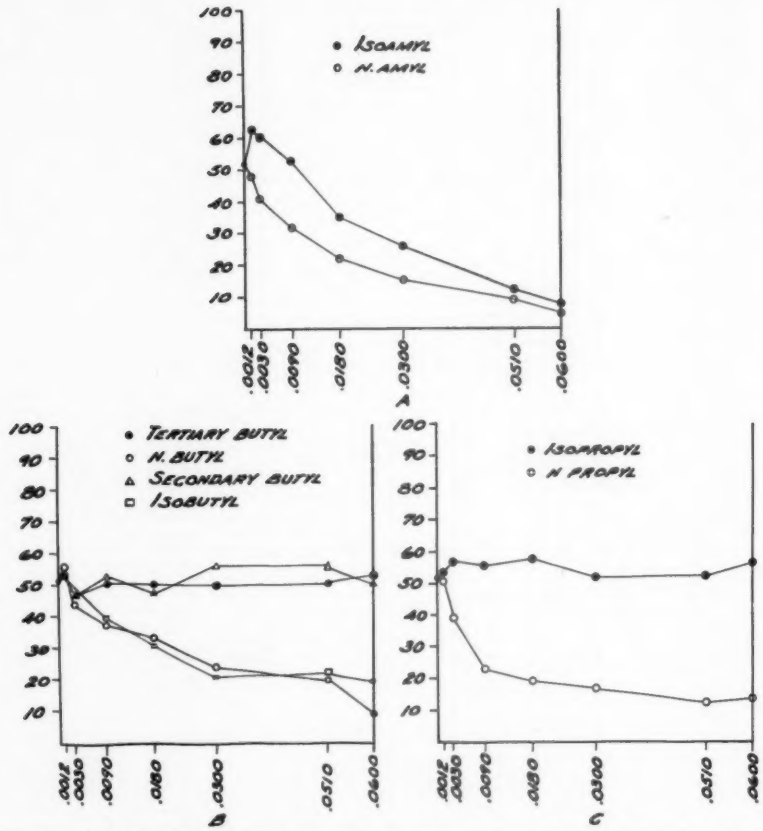

FIG. 4. Ash of soy-bean shoots grown in simple solutions of eight different alcohols. Ordinates represent percentages of ash for standard solution. Abscissas represent volume-molecular concentrations.

Growth in mixtures of alcohol and calcium nitrate

It has been found that mixtures of certain salts have an ameliorating effect with respect to toxicity as compared with growth of seedlings in solutions when each of the salts is used singly. The ion that exerts the most antagonistic effect seems to be calcium.

As previously stated, the author has attempted to determine the effect of a calcium salt on the growth of seedlings at different concentrations when the salt was mixed with certain normal alcohols—methyl, ethyl, propyl, butyl, and amyl.

TABLE IV

MOLECULAR CONCENTRATIONS OF SINGLE ALCOHOL SOLUTIONS REQUIRED TO PRODUCE EQUAL QUANTITIES OF ASH IN SHOOTS OF SEEDLINGS

RELATIVE GROWTH RATE	METHYL	ETHYL	N PROPYL	ISOPROPYL	N BUTYL	ISOBUTYL	SECONDARY BUTYL	TERTIARY BUTYL	N AMYL	ISOAMYL	N HEXYL
60		0.0006								0.0012	
50	0.0024	0.0354	0.0018		0.0018	0.003	0.0024	0.0024	0.0006	0.0102	
40		0.0486	0.003		0.0066	0.0084			0.0036	0.0156	0.0012
30		0.06	0.0066		0.0216	0.0192			0.0108	0.0246	0.0036
20			0.0162		0.051	0.0564			0.0210	0.0390	0.009
10			0.051		0.06				0.051	0.0546	.00216

In all instances but one, the toxic effect of these mixtures of alcohol and $Ca(NO_3)_2$ is greater than that of $Ca(NO_3)_2$ when used alone, or of the alcohol when used alone. The one exception was methyl alcohol and $Ca(NO_3)_2$. At concentrations approaching a total concentration of 0.03 M. there was evidence of antagonism as indicated by relative root elongation.

It will be observed from fig. 5 that the increased toxicity due to mixtures is much greater when one uses a very toxic alcohol, such as propyl or amyl. In case of these alcohols the general outline of the mixture curves resembles the curve of the alcohol used singly.

LOEW (**16**) stated that it was necessary for certain metal ions to combine in a definite ratio with the proteins of the plant in order to produce normal growth. It seems that in this instance there was no combination of alcohols and protoplasm, or, if there was, the resultant combination was inimical to proper growth. The alcohols seem also to have prevented $Ca(NO_3)_2$ from becoming inert; or, by preventing it from being adsorbed, subsequently prevented adsorption by the plasma membrane.

METHYL ALCOHOL AND CALCIUM NITRATE.—Observation of the growth allowed by mixtures of methyl alcohol and $Ca(NO_3)_2$ (fig. 5) reveals an example of antagonism. The root elongation in mixtures is higher than in solutions where $Ca(NO_3)_2$ was used alone, and in part greater than when methyl alcohol was used singly. However, high salt content and low alcohol content, or low salt content and high alcohol content of solutions afforded a medium for growth more favorable than the mixture. Only where the components of the mixture approach equality is there evidence of antagonism.

ETHYL ALCOHOL AND CALCIUM NITRATE.—By referring to table V it will be found that in the mixtures of ethyl alcohol and $Ca(NO_3)_2$, where the components were present in equal molecular quantities, the root elongation was slightly less than the root elongation when the same amount of $Ca(NO_3)_2$ was used alone. However, the root elongation when equal quantities of the components were employed was 22 per cent. less than the root elongation, when ethyl alcohol alone was used in the same concentration as was used in the mixture.

It seems that the root elongation in mixtures containing high proportions of $Ca(NO_3)_2$ and small proportions of ethyl alcohol was not much different from the root elongation in solutions containing high proportions of ethyl alcohol and small proportions of $Ca(NO_3)_2$.

NORMAL PROPYL ALCOHOL AND CALCIUM NITRATE.—From fig. 5, it is evident that throughout all concentrations there is a marked increase of toxicity as indicated by root elongation when the mixtures of propyl alcohol and $Ca(NO_3)_2$ are used. At no point on the graphs do the lines intersect. The growth in a 2 per cent. solution of 0.06 M. $Ca(NO_3)_2$ is 100 per cent. better than in a solution containing a mixture of 2 per cent. solution of 0.06 M. $Ca(NO_3)_2$ and 98 per cent. of 0.06 M. normal propyl alcohol; and

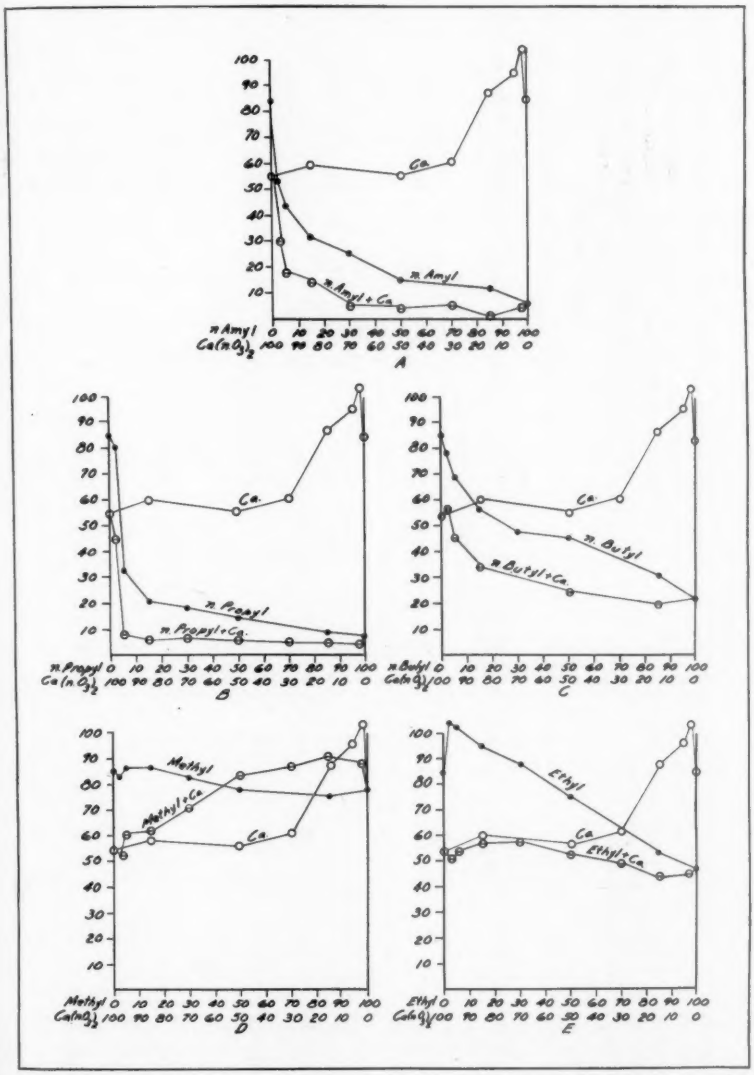

FIG. 5. Elongation of soy-bean roots in mixed solutions of alcohol and calcium nitrate, and in simple solutions of these alcohols and calcium nitrate in the concentrations in which they exist in the mixed solutions. Ordinates represent percentages of elongation for standard solution; abscissas represent molecular proportions.

TABLE V

GROWTH IN MIXED SOLUTIONS OF ALCOHOL AND CALCIUM NITRATE AS INDICATED BY ROOT ELONGATION; GROWTH VALUES REPRESENT THE AVERAGE OF FIFTY SEEDLINGS AND ARE EXPRESSED AS PERCENTAGES OF GROWTH FOR THE THREE-SALT STANDARD SOLUTION

PROPORTIONS OF 0.06 M.		METHYL	ETHYL	NORMAL PROPYL	NORMAL BUTYL	NORMAL AMYL
$Ca(NO_3)_2$	ALCOHOL					
100	0	54	54	54	54	54
98	2	52	51	45	57	30
95	5	60	53	8	45	18
85	15	61	57	6	34	14
70	30	71	58	7	30	5
50	50	81	53	6	25	4
30	70	85	49	5		5
15	85	91	44	5	20	1
2	98	89	46	4		4
0	100	78	48	7	22	6

GROWTH IN MIXED SOLUTIONS OF ALCOHOL AND CALCIUM NITRATE AS INDICATED BY RELATIVE ASH CONTENT OF SHOOTS; GROWTH VALUES REPRESENT THE AVERAGE OF FIFTY SEEDLINGS, AND ARE EXPRESSED IN PERCENTAGE OF ASH CONTENT OF SHOOTS OF SEEDLINGS GROWN IN THE THREE-SALT STANDARD SOLUTION

PROPORTIONS OF 0.06 M.		METHYL	ETHYL	NORMAL PROPYL	NORMAL BUTYL	NORMAL AMYL
$Ca(NO_3)_2$	ALCOHOL					
100	0	55	55	55	55	55
98	2	55	54	48	65	44
95	5	53	53	22	47	28
85	15	53	54	18	38	18
70	30	60	45	19	29	13
50	50	62	37	17	18	12
30	70	64	35	15		8
15	85	70	34	14	11	6
2	98	70	34	14		6
0	100	49	31	13	9	5

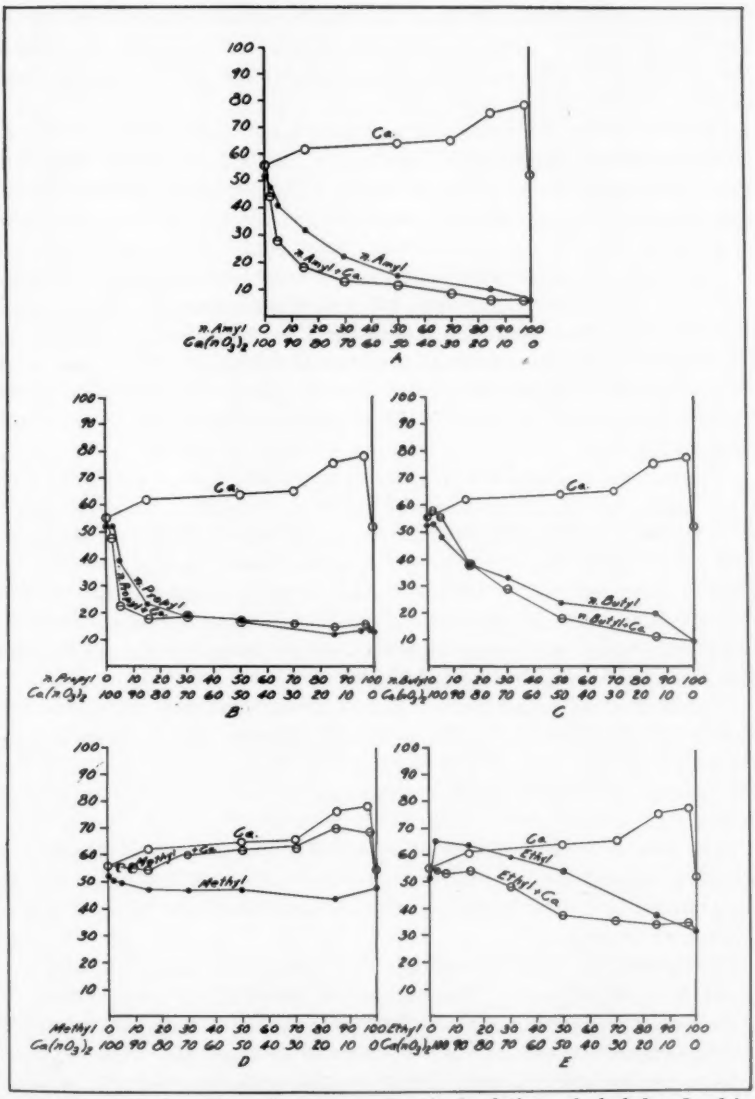

FIG. 6. Ash of soy-bean shoots grown in mixed solutions of alcohol and calcium nitrate, and in simple solutions of these alcohols and calcium nitrate in the concentrations in which they exist in mixed solutions. Ordinates represent percentages of ash for standard solution; abscissas represent molecular proportions.

a solution containing 2 per cent. of 0.06 M. normal propyl alcohol and 98 per cent. of 0.06 M. $Ca(NO_3)_2$ allows a growth that is 36 per cent. less than the growth in a solution containing 2 per cent.of 0.06 M. propyl alcohol singly.

NORMAL BUTYL ALCOHOL AND CALCIUM NITRATE.—The toxicity of mixtures of normal butyl alcohol and $Ca(NO_3)_2$ is found to be greater than when each of the components was used singly. This increased toxicity is not so pronounced as in the case of normal propyl alcohol, yet it is obvious as seen in fig. 5.

A solution containing the components 98 per cent. of 0.06 M. $Ca(NO_3)_2$ and 2 per cent. of 0.06 M. normal butyl alcohol allows a growth rate 22 per cent. less than does a solution containing 2 per cent. normal butyl alcohol used singly. The components 15 per cent. of 0.06 M. $Ca(NO_3)_2$ and 85 per cent. of 0.06 M. normal butyl alcohol allows a growth rate that is 68 per cent. less than the growth in a solution 15 per cent. of 0.06 M. $Ca(NO_3)_2$ solution used singly.

NORMAL AMYL ALCOHOL AND CALCIUM NITRATE.—Normal amyl, one of the most toxic alcohols used, when mixed with $Ca(NO_3)_2$ exerts a marked increase of toxicity as compared to the growth when each of the components was used singly. As the toxicity of normal amyl alcohol resembles the toxicity of normal propyl alcohol in some respects, so the increased toxicity due to mixing normal amyl alcohol and $Ca(NO_3)_2$ is comparable to the increased toxicity of mixtures of normal propyl alcohol and $Ca(NO_3)_2$ over solutions of the components used singly.

Throughout all concentrations this increased toxic effect prevails. The growth in a solution containing a mixture of 2 per cent. of 0.06 M. amyl alcohol and 98 per cent. of 0.06 M. $Ca(NO_3)_2$ is about 24 per cent. less than the growth in a single solution containing 2 per cent. of 0.06 M. amyl alcohol.

The growth allowed in a solution containing a mixture of 98 per cent. of 0.06 M. normal amyl alcohol and 2 per cent. of 0.06 M. $Ca(NO_3)_2$ was 100 per cent. less than in a single salt solution containing 2 per cent. of 0.06 M. solution of $Ca(NO_3)_2$.

The growth in a solution containing a mixture of $Ca(NO_3)_2$ and amyl alcohol was approximately the same as the growth in a single solution of hexyl alcohol, comparing solutions having equal molar quantities of alcohol.

COMPARISONS OF TWO CRITERIA OF GROWTH IN MIXTURES.—The difference between the growth in single alcohol solution and the growth in solutions containing alcohol and $Ca(NO_3)_2$ is usually less when the relative ash content is used as a criterion than when the root elongation is used.

There are some individual differences also which are noticeable. The degree of antagonism—increased growth in solutions of mixtures over that

in single solutions—is shown to be numerically higher when the criterion of ash weight is used as a basis of growth measurement. This feature seems evident when the difference in growth between that in single methyl alcohol solutions and growth in mixtures of methyl alcohol and $Ca(NO_3)_2$ is noted. On the other hand, when the ash criterion is used, the difference in growth in a single solution of $Ca(NO_3)_2$ and the growth in a solution containing a mixture of $Ca(NO_3)_2$ and methyl alcohol indicates an increased toxic effect. But when the rate of root elongation is used as a criterion the data indicates for the same instance, antagonism.

Another example where the two methods of measuring growth seem to vary is the case of normal butyl alcohol. The curves (fig. 6) indicate that there is little difference between the growth of seedlings in normal butyl alcohol used singly, and in mixtures of normal butyl alcohol and $Ca(NO_3)_2$. On the other hand, when the root elongation is the criterion (fig. 5) the growth rate appears to be considerably higher in the case of single solutions than in the corresponding mixture.

Summary

A study was made of elongation of soy-bean roots and the ash content of the shoots of these plants with simple solutions of calcium nitrate and the following alcohols: methyl, ethyl, normal propyl, isopropyl, normal butyl, isobutyl, secondary butyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl. The single solutions varied in concentration from 0.0012 M. to 0.06 M. With each total concentration nine different sets of alcohol proportions were tested. Soy-bean seedlings having roots with an initial length of about 8 mm. were placed in the culture solutions and grown in darkness. Each test was terminated when the roots in a standard solution had attained a length of 95 mm. Amounts of root elongation, expressed as percentages of the elongation in the standard solution were used as the basis for quantitative comparisons of the physiological effects of the culture solutions. Amounts of ash of shoots, expressed as percentages of the ash of shoots in standard solutions were also used. The principal results and conclusions follow:

1. With the exception of the less toxic alcohols, the root elongation and ash content of shoots were lower in single alcohol solutions than in distilled water. Elongation was inversely related to concentration, but no simple rule applicable to every case can be formulated regarding the relationship of toxicity to concentration over the range of concentrations tested.

2. Single solutions of ethyl alcohol and of calcium nitrate at low concentrations exert a stimulating influence for increasing growth. At a concentration of 0.0012 M. these afford a medium for growth better than that of a standard solution. This stimulating influence is less pronounced in the case of isobutyl and isopropyl alcohol.

3. When root elongation is the criterion for growth, the order of toxicity (from least to most toxic) for moderate retardation (0.003 M.) is as follows: isopropyl, methyl, ethyl, secondary butyl, calcium nitrate, normal butyl, isobutyl, tertiary butyl, normal amyl, normal propyl, isoamyl, and normal hexyl. For high retardation (0.06 M.) the order is as follows: methyl, isopropyl, secondary butyl, calcium nitrate, ethyl, normal butyl, isobutyl, normal propyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl.

4. When relative ash content is used as a criterion, the order of toxicity (from least to most toxic) for moderate retardation (0.03 M.) is as follows: calcium nitrate, secondary butyl, ethyl, isopropyl, tertiary butyl, methyl, isoamyl, normal butyl, isobutyl, normal propyl, normal amyl, normal hexyl. At high retardation (0.06 M.) the order is as follows: isopropyl, calcium nitrate, tertiary butyl, secondary butyl, methyl, ethyl, isobutyl, normal propyl, normal butyl, isoamyl, amyl, normal hexyl. Relatively more ash was found in shoots grown in a solution of 0.0012 M. $Ca(NO_3)_2$ than in those grown in solutions of higher concentration.

5. When seedlings were grown in mixtures of calcium nitrate and methyl alcohol there was evidence of slight antagonism—the solutions were not quite as toxic as when the components were used singly. When the individual alcohols, ethyl, normal propyl, normal butyl, and normal amyl were used in mixtures with calcium nitrate, the toxic effects were increased—the toxic effect of the mixtures was greater than the toxic effect when the components were used singly. This increase was most pronounced when normal amyl was used in mixtures with calcium nitrate.

6. The toxicity of alcohols does not seem to increase regularly with increased number of carbon atoms in the molecules. Not in all instances is the normal alcohol more toxic than the iso-alcohol.

7. Equations are given for computing approximate growths.

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CHANGES IN THE COMPOSITION OF THE TOMATO PLANT ACCOMPANYING DIFFERENT STAGES OF YELLOWS[1,2]

MICHAEL SHAPOVALOV AND HENRY A. JONES

Purpose of this investigation

In 1927 ROSA[3] published data which show that tomato yellows is characterized by definite changes in chemical composition in all parts of the host plant. His material was collected from healthy as well as from the affected plants showing different degrees of severity of the disease. Not only were the amounts of certain constituents in healthy plants different from those in blighted plants, but there was also a considerable variation in the composition of the different diseased samples. However, it was not apparent that this variation was definitely associated with different stages of the disease, since the samples obtained from different plants and on different days were not strictly comparable. Furthermore, the identification of the disease in the case of ROSA's material was based exclusively on external appearances of the plants used. There was no knowledge of either the previous history or the etiology of these abnormal appearances. It was not certain that the results obtained with this material could likewise be applied to the seemingly identical pathological phenomenon induced by the curly-top virus through the medium of the beet leafhopper, *Eutettix tenellus* Baker.

It still remained to be shown, therefore, whether the peculiar chemical changes observed in connection with tomato yellows accumulate in the plant gradually as the diseased conditions become more and more severe, and whether the field phenomenon known as tomato yellows is identical in its inner processes as well as in its extrinsic symptomatology with the disease caused by the curlytop virus. To show this, it was necessary to use artificially inoculated material and to analyze different stages of the disease as represented by different plants on the same day, as well as various stages of yellows shown by the same plant on different dates. In addition to this, it appeared desirable to ascertain whether there is a difference in the response to the yellows infection between the known resistant and the susceptible varieties. For this purpose several resistant strains developed

[1] Tomato yellows is the same disease which in the previous literature was designated as western yellow blight. See U. S. Dept. Agr. Misc. Publ. no. 13.

[2] Joint contribution from the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., and the Division of Truck Crops, University of California, Davis, California.

[3] ROSA, J. T. Chemical changes accompanying the western yellow blight of tomato. Plant Physiol. 2: 163–169. 1927.

by Dr. J. W. LESLEY were used. It was Dr. ROSA's intention to continue the work along the lines indicated above, but these plans were left unfulfilled because of his untimely death. The writers considered it very urgent to complete this phase of the work. The inoculations were made and the samples preserved at the Citrus Experiment Station, Riverside, California; the analyses were made in the division of Truck Crops, Davis, California.[4]

Methods employed

To obtain the diseased samples for analytical purposes a number of plants were inoculated by allowing viruliferous beet leafhoppers to feed on these plants. The following method of inoculation was followed: A celluloid cell, about 5 inches long and 2.5 inches in diameter, with muslin ends, was placed over the tip of each plant, and several insects injected into each cell. The number of insects used in different series of inoculations varied from 5 to 20, but each plant in a given series received the same number. Likewise the period during which the insects were allowed to feed on the plant varied in different series from 2 to 6 days, but it was the same for every plant in a given series. One series was inoculated in November, 1927 (table I), one in the spring of 1928 (table II) and one in the summer of 1928 (table III). When possible, samples were taken at different intervals from the same inoculated plants, as well as from the same healthy plants used for checks.

The plants used can be identified by their numbers in each series. In the first series inoculated plants nos. 2 and 3 were omitted and a new plant no. 5 was introduced, for the reason that the former did not develop the disease while the latter did. Plant no. 1 in the second series was used in its entirety during the first sampling on June 16. No substitution for the check plants was made in any of the series. Samples in series no. 3 (table III) were taken in Dr. J. W. LESLEY's field. The following types of material were selected: (a) Uninoculated healthy plants; (b) plants inoculated, but showing no symptoms of yellows; (c) plants showing an early or medium stage of yellows, represented by a complex of symptoms preceding distinct yellowing, and (d) plants showing a late stage of yellows, when the characteristic sulphur-yellow discoloration is well marked. Detailed descriptions of these samples and the results of the analyses are given in Tables I, II, and III.

Fifty-gram samples were collected shortly before noon. They were immediately preserved in boiling 95 per cent. alcohol. After drying the solid portion of the sample, and grinding, extraction was completed with 50 per cent. alcohol. Carbohydrates were determined by the method used

[4] Acknowledgment is made to HAROLD I. SIPMAN for performing the analytical work and to F. SIDNEY BEECHER for assistance in preparing the samples.

TABLE I

COMPOSITION OF INOCULATED TOMATO PLANTS IN DIFFERENT STAGES OF THE DEVELOPMENT OF YELLOWS IN COMPARISON WITH UNINOCULATED HEALTHY CHECKS. SERIES 1, INOCULATED NOVEMBER 9, 1927. ALL PLANTS EXCEPT CHECKS WERE INOCULATED. RESULTS EXPRESSED IN PERCENTAGE OF FRESH WEIGHT

PLANT NUMBER	DATE OF COLLECTING SAMPLES	CONDITION OF MATERIAL	DRY MATTER	DEXTROSE	SUCROSE	STARCH	NITROGEN			
							SOLUBLE	INSOLUBLE	TOTAL	PER CENT. SOLUBLE
		Leaves								
1	11–17–27	Check, healthy[a]	10.0	0.16	0.00	0.17	0.034	0.286	0.320	10.7
	12–12–27	Check, healthy[a]	10.8	0.25	0.18	0.41	0.074	0.244	0.318	23.2
	1–18–28	Check, healthy[a]	13.3	0.31	0.38	0.59	0.049	0.438	0.487	10.0
2	11–17–27	Apparently healthy	11.6	0.18	0.22	0.20	0.124	0.237	0.361	34.4
3		Apparently healthy	14.3	0.24	0.24	0.98	0.063	0.453	0.516	12.3
4	11–17–27	Apparently healthy	14.8	0.24	0.23	1.04	0.073	0.374	0.447	16.3
	12–12–27	Early medium stage of yellows[b]	16.9	0.61	0.34	1.79	0.071	0.424	0.495	14.3
	1–18–28	Late stage of yellows	16.9	1.42	0.53	1.74	0.071	0.329	0.400	17.7
5	12–12–27	Early medium stage of yellows	16.5	0.68	0.40	1.68	0.063	0.373	0.436	14.5
	1–18–28	Late stage of yellows	16.9	1.33	0.63	1.15	0.102	0.367	0.469	21.8
		Stems								
1	11–17–27	Check, healthy[a]	3.6	0.57	0.16	0.05	0.178	0.120	0.298	59.6
	12–12–27	Check, healthy[a]	8.7	0.68	0.24	0.16	0.090	0.112	0.202	44.6
	1–18–28	Check, healthy[a]	8.7	0.39	0.29	0.16	0.095	0.127	0.222	42.8
2	11–17–27	Apparently healthy	7.7	0.36	0.13	0.05	0.151	0.120	0.271	55.7
3	11–17–27	Apparently healthy	7.4	0.48	0.17	1.01	0.114	0.101	0.215	53.1
4	11–17–27	Apparently healthy	8.4	0.60	0.19	0.20	0.112	0.110	0.222	50.4
	12–12–27	Early medium stage of yellows[b]	13.6	1.54	0.79	0.69	0.041	0.139	0.180	22.9
	1–18–28	Late stage of yellows	15.8	1.17	1.15	1.98	0.002	0.186	0.188	1.1
5	12–12–27	Early medium stage of yellows	13.6	1.42	0.89	1.03	0.066	0.141	0.207	31.9
	1–18–28	Late stage of yellows	12.3	1.30	1.09	1.32	0.001	0.122	0.123	0.9

[a] Same check plant.

[b] Only plant number 4 showed symptoms of the disease, while plants 2 and 3 did not, and were discarded.

TABLE II

COMPOSITION OF INOCULATED TOMATO PLANTS IN DIFFERENT STAGES OF THE DEVELOPMENT OF YELLOWS IN COMPARISON WITH UNINOCULATED HEALTHY CHECKS. SERIES 2, INOCULATED JUNE 28, 1928. ALL PLANTS EXCEPT CHECKS WERE INOCULATED. RESULTS EXPRESSED AS PERCENTAGE OF FRESH WEIGHT

PLANT NUMBER	DATE OF COLLECTING SAMPLES	CONDITION OF MATERIAL	DRY MATTER	DEXTROSE	SUCROSE	STARCH	NITROGEN			
							SOLUBLE	INSOLUBLE	TOTAL	PER CENT. SOLUBLE
		Leaves								
		A								
1*	7–16–28	Yellows in advanced stage, very chlorotic	17.3	1.29	0.57	3.43	0.092	0.295	0.387	23.9
2	7–16–28	Yellows in early stage, leaves rigid, but not very chlorotic	15.8	0.57	0.30	3.23	0.029	0.127	0.156	18.6
	7–30–28	Advanced stage of yellows	16.3	1.37	0.34	4.47	0.017	0.579	0.596	2.9
3	7–16–28	Similar to no. 2 on this date, but a little more advanced; slightly more discoloration	17.7	0.54	0.29	3.44	0.063	0.339	0.402	15.7
	7–30–28	Slightly more advanced than no. 2 on this date	17.6	1.22	0.59	3.53	0.001	0.558	0.559	0.2
4	7–16–28	Apparently healthy	12.9	0.30	0.26	1.31	0.068	0.423	0.491	13.8
	7–30–28	Still healthy	13.3	0.25	0.31	0.68	0.007	0.389	0.396	1.8
5	7–16–28	Apparently healthy	12.4	0.26	0.17	1.11	0.049	0.321	0.370	13.2
	7–30–28	Still healthy	12.7	0.18	0.34	1.31	0.056	0.405	0.461	12.1
6	7–16–28	Check. Healthy	11.8	0.30	0.25	0.52	0.074	0.384	0.458	16.1
	7–30–28	Check. Healthy	12.0	0.36	0.22	1.69	0.063	0.446	0.520	12.4
		B								
9*	7–30–28	Check. Healthy	13.0	0.38	0.35	1.27	0.054	0.375	0.429	12.5
10	7–30–28	Check. Healthy	12.6	0.31	0.37	1.08	0.056	0.375	0.431	13.0
8	7–30–28	Between medium and advanced stage of yellows	15.2	0.57	0.27	1.89	0.073	0.289	0.362	20.2
7	7–30–28	Advanced stage of yellows	17.3	0.83	0.29	3.66	0.056	0.283	0.339	16.5

* Numbers 1 to 6 in this table group A are the Stone variety; 7 to 10 group B, resistant selections, no. 0.012 of Dr. J. W. LESLEY.

TABLE II—*Continued*

Plant number	Date of collecting samples	Condition of material	Dry matter	Dextrose	Sucrose	Starch	Nitrogen			
							Soluble	Insoluble	Total	Per cent. soluble
		Stems								
		A								
1*	7–16–28	Yellows in advanced stage	13.5	1.44	1.31	0.54	0.152	0.159	0.311	48.9
2	7–16–28	Yellows in early stage, leaves rigid, but not very chlorotic	11.2	1.30	0.69	0.65	0.080	0.129	0.209	38.3
	7–30–28	Advanced stage of yellows	13.0	1.18	1.29	1.23	.....	0.143	.....	—
3	7–16–28	Similar to no. 2 on this date but a little more advanced; slightly more discoloration	10.9	0.93	0.55	0.52	0.092	0.125	0.217	42.5
	7–30–28	Slightly more advanced stage of yellows than no. 2 on this date	14.5	0.99	1.33	0.79	0.156	0.157	0.313	49.8
4	7–16–28	Apparently healthy	7.5	0.55	1.16	0.16	0.044	0.083	0.127	34.5
	7–30–28	Still healthy	9.4	0.55	0.26	0.25	0.054	0.098	0.152	35.5
5	7–16–28	Apparently healthy	8.3	0.38	0.17	0.16	0.083	0.094	0.177	46.8
	7–30–28	Still healthy	8.7	0.60	0.21	0.15	0.080	0.100	0.180	44.5
6	7–16–28	Check. Healthy	8.4	0.45	0.16	0.20	0.092	0.112	0.204	45.2
	7–30–38	Check. Healthy	8.3	0.73	0.33	0.18	0.102	0.086	0.188	54.3
		B								
9*	7–30–28	Check. Healthy	10.6	0.70	0.38	0.13	0.051	0.097	0.148	34.4
10	7–30–28	Check. Healthy	9.4	0.63	0.25	0.16	0.075	0.097	0.172	43.7
8	7–30–28	Between medium and advanced stage of yellows	13.6	0.83	0.91	0.61	0.139	0.155	0.294	47.3
7	7–30–28	Advanced stage of yellows	14.8	0.84	0.97	0.95	0.119	0.137	0.256	46.6

* Numbers 1 to 6 in this table group A are the Stone variety; 7 to 10 group B, resistant selections, no. 0.012 of Dr. J. W. Lesley.

by BISSON and SEWELL[5]—sucrose after inversion with invertase ($\frac{1}{3}$ cc. in 100 for 24 hours), and starch after autoclaving for one hour and digesting with a buffered solution of taka-diastase (pH = 4.66) for 24 hours. A blank was run using soluble starch, and the factor from that recovery was used to calculate the results of the other starches. Total nitrogen was determined by the official Gunning method. Nitrogen was determined separately on aliquots of the insoluble and alcohol-soluble portions of each sample.

Results of the analyses

The results obtained with artificially inoculated tomato plants are in general accord with the data published by ROSA. The composite samples listed in table III gave the closest parallel to ROSA's findings which likewise were based on composite samples. The behavior of individual plants, however, shows considerable variation, although the main process in the affected plants appears to be the same as indicated by composite samples.

Dry matter.—The percentage of dry matter always appeared to be greater in both leaves and stems of the plants inoculated with viruliferous beet leafhoppers, after the infected plants developed external symptoms of yellows, than in those of healthy checks. This is true of the tested resistant selections as well as of the susceptible variety, Stone. When inoculated plants failed to develop visible symptoms of the disease the amount of dry matter was about the same as in corresponding checks, as shown by plants 4 and 5 in table II. Advanced stages of yellows, whether in one and the same plant or in different plants, in the case of susceptible as well as resistant strains, were accompanied by larger amounts of dry matter than medium or initial stages. An exception is presented by leaves of plants 2 and 3 in table II, which showed practically unchanged percentage of dry matter at two different samplings made two weeks apart, although in both cases higher than in checks on same dates. These plants also revealed an increase in total and insoluble nitrogen and a decrease of its soluble portion. During the winter months (series 1) the percentage of dry matter increased in the healthy check plants also, although this increase appeared to be limited to the leaves and was not apparent in the stems.

Carbohydrates.—Leaves and stems of the inoculated plants of all the tested strains showed decidedly higher carbohydrate contents than in the case of uninoculated checks on the same dates. The results obtained with composite samples were again most strikingly consistent, especially with stems of these samples. Plants showing advanced symptoms of yellows, as a rule appeared to be more abundantly supplied with carbohydrates than those which were still in earlier stages of the disease, although there were

[5] BISSON, C. S., and SEWELL, J. GORDON. The estimation of cuprous oxide produced in sugar analysis. Jour. Assoc. Offic. Agr. Chem. 10: 120–124. 1927.

TABLE III

COMPOSITION OF YELLOWS RESISTANT AND YELLOWS SUSCEPTIBLE, INOCULATED AND DISEASED AS WELL AS UNINOCULATED HEALTHY, TOMATO PLANTS. ALL SAMPLES IN THIS SERIES (3) ARE COMPOSITE, COLLECTED ON SEPTEMBER 5, 1928. RESULTS EXPRESSED AS PERCENTAGE OF FRESH WEIGHT

SAMPLE NUMBER	CONDITION OF MATERIAL	DRY MATTER	DEXTROSE	SUCROSE	STARCH	NITROGEN			
						SOLUBLE	INSOLUBLE	TOTAL	PER CENT. SOLUBLE
	Leaves								
1*	Healthy dwarf (resistant) no. .006	15.2	0.37	0.30	2.04	0.039	0.313	0.352	11.1
2	Inoculated and diseased " "	18.1	1.58	0.62	2.81	0.061	0.233	0.294	20.7
3	Healthy standard (resistant) no. .018	15.4	0.34	0.20	1.25	0.039	0.260	0.299	13.0
4	Inoculated and diseased " " "	16.3	0.75	0.30	1.90	0.044	0.220	0.264	16.6
5	Healthy standard (susceptible) no. .014	17.1	0.40	0.45	2.05	0.039	0.277	0.316	12.3
6	Inoculated and diseased " " "	.....	.....	.....	3.86	.....	0.221	.....	.....
	Stems								
1*	Healthy dwarf (resistant) no. .006	14.2	1.01	1.46	0.56	0.032	0.089	0.121	26.4
2	Inoculated and diseased " "	19.0	1.48	2.61	2.67	0.092	0.134	0.226	40.7
3	Healthy standard (resistant) no. .018	14.7	1.00	0.55	0.59	0.054	0.091	0.145	37.2
4	Inoculated and diseased " " "	17.7	1.47	1.83	1.46	0.073	0.116	0.189	38.7
5	Healthy standard (susceptible) no. .014	17.4	0.97	1.26	1.28	0.034	0.105	0.139	24.5
6	Inoculated and diseased " " "	20.6	1.14	2.25	2.63	0.074	0.097	0.171	43.2

* In numbers 1 and 2, a composite of 3 plants was used for a sample. In numbers 3 to 6, a composite of 4 plants was used.

a few insignificant exceptions. While sugars as well as starch tend to accumulate in the inoculated and affected plants, this process is particularly pronounced and constant in the case of starch.

Nitrogen.—Changes in the nitrogen contents in connection with the development of yellows in artificially inoculated plants were somewhat variable although the general direction in which these changes proceed may still be recognized. There was a tendency for total nitrogen to decrease in the affected leaves at the expense of the insoluble fraction, as is well shown by the composite samples of series 3 (table III) and by the resistant individual plants in series 2 (table II, B). Stems of the same plants, on the contrary, showed a decided tendency for total nitrogen to increase. The data obtained with leaves and stems of the affected susceptible plants are somewhat contradictory. The same lack of complete uniformity may be observed with respect to the percentage of soluble nitrogen. It is higher in the composite samples of series 3 and the resistant individuals of series 2, both in leaves and stems, but the susceptible variety, Stone, showed different tendencies in different plants with no distinct difference between the leaves and the stems.

Internal vs. external changes.—The composition of individual plants in different stages of the development of yellows shows very clearly that the accumulation of dry matter, starch and sugars, is progressive and gradual, and as a rule corresponds to the severity of the external symptoms of the disease. With some plants this internal process is more marked in the leaves, with others, in the stems.

With respect to nitrogen, our results do not indicate any gradual or progressive changes which are consistent. Plant 4 in series 1 showed a decrease in both total and insoluble nitrogen in leaves, and their increase in stems, whereas quite opposite changes seemed to take place in plant 5; the percentage of the soluble fraction increased in the leaves of both plants, while showing a tremendous decrease in the stems. The predominating tendency in series 2 was more apparent than in series 1. With the exception of sample 4, there was a decided increase in total nitrogen and its insoluble fraction in the leaves and stems. Sample 4 showed a decrease of these constituents in the leaves and their increase in the stems, quite like plant 4 in series 1. The most surprising phenomenon was the decrease in the percentage of soluble nitrogen in the leaves, particularly in the case of samples 2, 3 and 4. With one exception, there was an increase of this fraction in the stems.

It is difficult to explain the striking decrease of soluble nitrogen in the leaves of plants 2, 3 (inoculated and diseased) and 4 (inoculated but not externally diseased) of series 2 on the basis of the present limited knowledge of internal changes that accompany the phenomenon known as tomato

yellows. It is possible that this decrease was due to the inactivation of virus, and the resumption of growth by the inoculated plants. This renewed growth activity may account for the decrease in the percentage of nitrogen in the soluble fraction.

Conclusions

1. Chemical changes accompanying external symptoms of tomato yellows produced by artificial inoculations with curlytop virus are essentially identical with those which accompany a similar complex of symptoms observed in the field and formerly known under the name of western yellow blight and other synonyms.

2. Among these changes the increase of dry matter and the accumulation of starch and sugars in the leaves as well as in the stems of the inoculated and naturally affected plants appeared to be constant and should be regarded as a condition characteristic of this virus malady.

3. Changes in the amounts of nitrogen are variable. There is often a decrease of total nitrogen, particularly in the leaves, at the expense of its insoluble fraction, with an increase in the percentage of the soluble portion. However, these changes are not constant and therefore may not be typical for tomato yellows.

4. The carbohydrate accumulation in the same plant is progressive, and larger amounts of these constituents are definitely connected with more severe external symptoms.

5. Resistant strains show essentially the same response to the yellows infection as do susceptible strains when composite samples taken on the same day are compared: A definite increase in the carbohydrate contents and an apparent tendency toward the increase in the soluble nitrogen and the decrease in the total and the insoluble portions in the leaves and *vice versa* in the stems, with some exception in the composite samples.

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THE FOURTH PACIFIC SCIENCE CONGRESS

E. M. HARVEY

(WITH SEVEN FIGURES)

The Fourth Pacific Science Congress was held in Batavia and Bandoeng, Java, May 16 to 25, 1929, under the auspices of the Netherlands Indies Science Council acting as a member of the Pacific Science Association. The Netherlands East Indies government cooperated with the Council in every way to assure the success of the Congress.

Overseas delegates were guests of the Netherlands East Indies government and Science Council, not only during the actual duration of the meetings, but also for all the numerous excursions provided from May 12 to June 5, throughout Java and neighboring islands. In his address which officially opened the meeting in Batavia, His Excellency, the Governor General of the Netherlands Indies, pleasantly expressed the hope that the American delegates would experience a "Dutch treat" in a sense otherwise than the one in which the term is often used "rather ungraciously to Dutch hospitality." It may be said with full assurance that His Excellency's hope was completely realized! Space will not permit so much as mention of the numerous interesting and sometimes highly instructive entertainments offered the delegates. Scarcely more than mention can be made of the principal excursions of interest to botanists and agriculturists. It may be added in passing, that the special scientific excursions were probably of equal, and sometimes of greater value than the meetings themselves. When one considers how full of treasures of scientific interest is that region of the world, it is not difficult to understand the enthusiasm of the delegates for studying nature in the East Indies first hand. The administration of the Congress anticipated this desire and provided for it most generously.

In attendance were 191 delegates (members) and perhaps 75 to 100 participants. Fig. 1* shows the group of delegates and participants and fig. 2, the Executive Committee of the Congress. Twenty-four countries were represented. Among the fifty or more delegates who may be classified as Botanists, a few will be mentioned as follows: Dr. D. VAN LEEUWEN, Director of the Botanical Gardens, Buitenzorg; Dr. F. VON FABER, Director of the Treub Laboratory, Buitenzorg; Dr. M. MYOSHI, Emeritus Professor of Botany, Tokyo Imperial University; Dr. F. A. F. C. WENT, Professor of Botany, University of Utrecht, and President of the Royal Academy of Sciences, Amsterdam; Dr. C. J. SKOTTSBERG, Director, Botanic Gardens, Gothenburg, Sweden; Dr. E. J. GODDARD, Dean of Agriculture,

* The figures are copied from illustrations furnished by the Congress.

University of Queensland, Brisbane; Dr. H. HOTTORI, Director, Tokugawa Biological Institute, Tokyo; Dr. W. A. SETCHELL, University of California; and Dr. F. V. COVILLE, U. S. Department of Agriculture. These are but a few of the distinguished members of the Congress, and other equally distinguished technical agriculturists and biochemists were in attendance. At least one should mention the name of Dr. O. DE VRIES, Director of the Rubber Experiment Station, Buitenzorg, and President of the Science Congress. Three members of the American Society of Plant Physiologists were present; namely, Dr. K. KORIBA, Professor of Botany, Kyoto Imperial University; Dr. G. A. C. HERKLOTS, University of Hong Kong; and the writer.

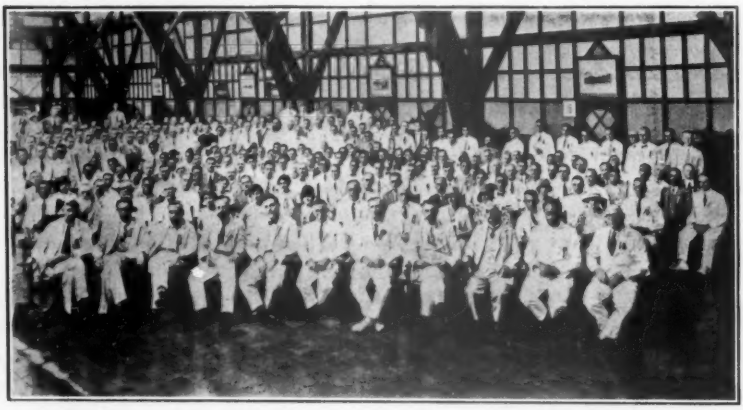


FIG. 1. Delegates and participants to the Fourth Pacific Science Congress, Bandoeng, Java, May, 1929.

The meetings were opened officially in Batavia, May 16, but the scientific program did not commence until May 18 in Bandoeng, where the meetings continued to May 25. Bandoeng was regarded as a more favorable location for the meetings than Batavia, on account of its higher altitude (2100 ft.) and consequent lower temperature.

One special feature of the program were the several symposia on topics, some of which were of general interest, and others of interest only to specific groups. The "case of Krakatau" and "Protection of Nature around the Pacific" are examples of the programs of general interest, while among those of more specific interest in the field of Botany and Agriculture, may be named the following: Rice problems, breeding and selection of certain crop plants, including rubber, sugar cane, coffee, etc.; forestry problems; soil technology; and plant geography.

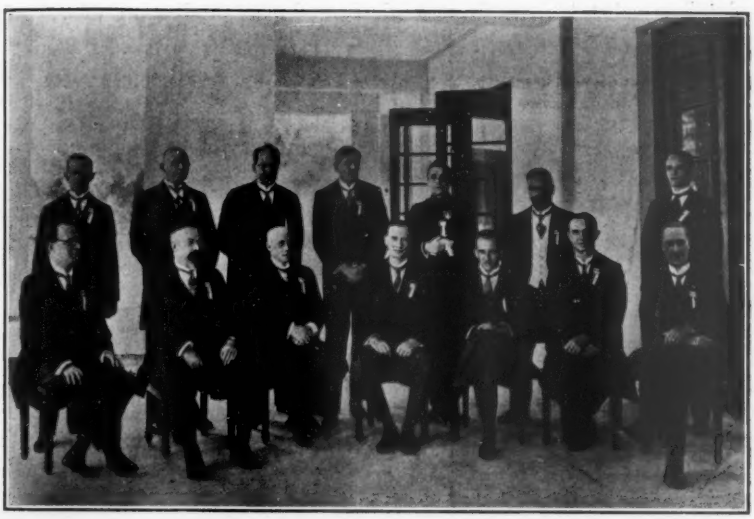


FIG. 2. Executive Committee of the Congress.

Standing, left to right: Dr. C. E. STEHN, Chief, Vulcanological Survey; Dr. J. STROOMBERG, Chief, Division of Commerce, Department of Agriculture, Industry, and Commerce; Dr. W. F. GISOLF, Petrographer, Geological Survey; Dr. S. W. VISSER, subdirector, Royal Magnetic and Meteorological Observatory; Dr. H. J. T. BYHMER, Military Surgeon and Anthropologist; Dr. K. W. DAMMERMANN, Chief, Zoological Museum and Laboratory, Buitenzorg; Dr. J. T. WHITE, Chief, Pedological Institute and General Agricultural Experiment Station.

Sitting, left to right: Dr. H. M. HIRSCHFIELD, Officer, Java Bank; Dr. W. M. DOCTERS VAN LEEUWEN, Director Buitenzorg Botanical Gardens; Dr. J. CLAY, Professor of Physics, Technical Faculty, Batavia; Dr. O. DE VRIES, Director, Rubber Experiment Station, and Professor of Chemistry on the Medical Faculty, Batavia (President of the Fourth Pacific Science Congress); Dr. H. J. LAM, Herbalist, Botanic Gardens, Buitenzorg; A. C. DE JONG, Director, Geological Survey; and Dr. J. J. B. DEUSS, Director Tea Experiment Station, Buitenzorg.

Rice is of so vast importance to the Eastern countries, that its problems were allowed considerable space on the program. In Java alone, the rice production is about 7¼ million tons per annum, grown on 8,000,000 acres. While even in Java the rice growing is entirely in the hands of the natives, the Dutch experiment stations attempt to give the industry every possible aid in improving varieties and cultural practices. The symposium on rice problems was opened by an extensive report on the economic situation by Mr. M. B. SMITS of the Division of Agricultural Economics of the Netherland East Indies, but the other papers and discussions were concerned mostly with such questions as fertilizers, the relation of temperature and

irrigation water to growth and yield, and the production of superior varieties. Fig. 3 shows rice fields near Bandoeng.

At the present time, all the rubber in the East Indies is derived from *Hevea braziliensis,* or rather varieties developed from it. Extensive investigations are being carried on in the breeding and selection of superior varieties; physiology of latex secretion; methods of tapping; preparation of the sap and its chemical composition; methods of propagation; and the combatting of pests. As a part of the selection work, records of individual tree yields are kept on all estates, and as soon as any tree shows unusual performance, it is officially recorded as a "mother tree," that it may serve later as material for further improvement.

Papers of the above nature were presented also for such crops as coffee, tea, cocoanuts, oil palms, and sugar cane.

The work on sugar cane seemed of especial value. By cultural improvements and the development of resistant varieties, the "sereh" disease has been practically conquered. In the breeding experiments aimed at the production of disease resistant and high yielding varieties, it is interesting to note that there appears to be a correlation between the number of chromosomes and the size, vigor, and sugar content of the cane.

In Plant and Animal Geography, the most popular discussions revolved around the history and development of the flora and fauna of the Krakatau Islands since the great eruption in 1883. The popularity of such discussions was greatly augmented by the delightful excursion which had been made to Krakatau, May 12 and 13. Fig. 4 shows the east side of the island, and its coastal vegetation.

The "Wallace-Weber line" drew some fire, but in the end it seemed that no distinct damage was done it.

A very interesting paper was read by Dr. VON FABER, Director of the Treub Laboratory on the subject: "The Physiology of the Mangroves." A careful study had been made on these plants, both *in situ* and in experimental culture in the laboratory. The result was a detailed record of the fluctuations of the environment and the internal adjustments of the plants to those fluctuations. When a plant is removed from a nutrient solution, with addition of 2–3 per cent. NaCl to one with 8 per cent. NaCl, there is an impediment to the "suction" of the roots, so that a small deficit arises between absorption and transpiration. The water tissues begin to get thin, while assimilation leaves remain turgescent. When the latter conditions remain unchanged, transpiration is greatly reduced by the closing of stomata. The roots begin their regulating activities, the deficiency is wiped out, and transpiration becomes normal. The "saltstorers" (*e.g., Avicennia officinalis*) make this adjustment in one to two hours, and the "non-saltstorers" (*e.g., Rhizophora spp.?*) in four to five hours. When the plant is

FIG. 3. Irrigated rice fields (Sawahs) near Bandoeng, Java.

FIG. 4. East side of Rakata or Krakatau Island in the Sunda Strait. The island is 2300 ft. high, and has reforested since 1883 to the 2000 ft. line. The northwest side of this peak is the precipitous cliff of Krakatau which is always shown in the geological photographs.

returned to a 3 per cent. NaCl solution, a surplus of water accumulates and, just as at high tide, the leaves of the non-saltstorers take on a glassy appearance. But the saltstorers, through their rapid root regulation and secretion, quickly adjust themselves. Osmotic pressure is greatest at low tide at which period the leaves of *Rhizophora* have shown 148.4 atmospheres and the roots 98.6 atmospheres. The respective values for *Avicennia* were 162.2 and 96.0 atmospheres. At the end of high tide *Rhizophora* showed 77.8 and 45.4 atmospheres for leaves and roots respectively. Correspondingly, *Avicennia* showed 82.0 and 50.3 atmospheres. Experimentally *Avicennia* could be induced to develop 205 atmospheres in the leaves, with transpiration still "very liberal."

Regarding the scientific excursions of the Congress, much might be written, and it requires considerable restraint to pass them by with a mere mention of those of special interest to Botanists and Agriculturists, as follows: Krakatau Islands in the Sunda Strait; Coral Islands in Batavia Bay; the famous Botanical Gardens and laboratories (see fig. 5 for the famous Treub Laboratory) in Buitenzorg; the high altitude Botanical Garden and virgin tropical rain forest at Tjibodas on Mt. Gede, fig. 6; to the crater of the active volcano, Mt. Tankoeban Prahoe, near Bandoeng; Mangrove, swamps of the Kinderzee and Noesa Kambangan on the south coast of Java near Tjilatjap—on Noesa Kambangan is also a low altitude tropical rain forest, which is the home of the huge parasitic flower *Rafflesia patma*, fig. 7; to Madoera island off the northeast coast of Java; to the Tengger mountains at Tosori, to visit the "Tjemara" (*Casuarina junghuniana*) forests and the active volcano, Mt. Bromo; and the marvelous island of Bali—this excursion was not strictly Botanical!

The Agriculturists were given opportunity to visit several of the principal experiment stations of Java of which there are perhaps eighteen. About half of these are privately endowed. As a rule, each of the experiment stations concerns itself with but one, or only a very few products. Some of these institutions are prepared in a splendid way, both in personnel and laboratory equipment, to carry out their aims. The Sugar Experiment Station at Pasoeroean, for example, has an annual income of over $500,000.00. This is a large sum for one experiment station and a single agricultural product, but that the investigational staff is accomplishing excellent results is indicated by the experimental work on sugar cane previously referred to, and very clearly by the fact that the sugar yield in Java has gone steadily upward to the present average of 14,000 lbs. per acre. This figure stands in striking contrast to the old average in Java of 1,700 lbs. and the present average in Louisiana of only 1,300 lbs. per acre.

Another very important phase of the excursions for the Agriculturists was the opportunity to inspect numerous large estates of Cinchona, rubber, tea, coffee, teak, and other crops.

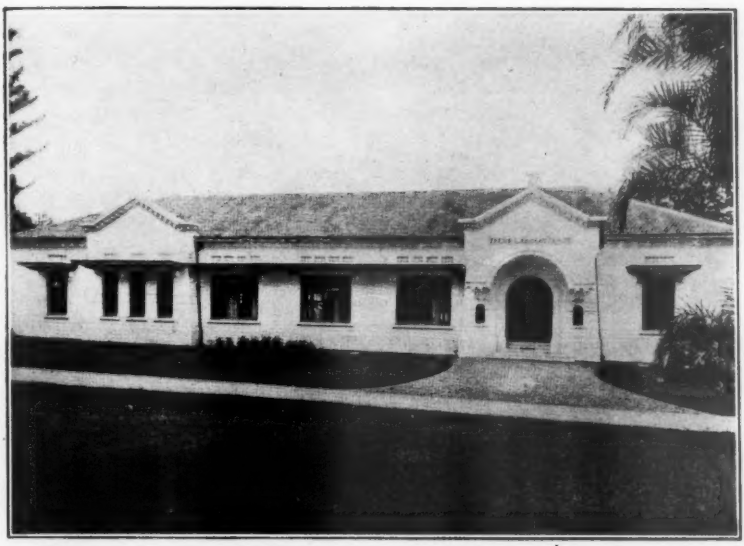


FIG. 5. The Treub Laboratory, Buitenzorg.

FIG. 6. Visitors' Laboratory, Botanical Gardens, Tjibodas, at altitude of nearly 5000 ft. on Mt. Gede, Java.

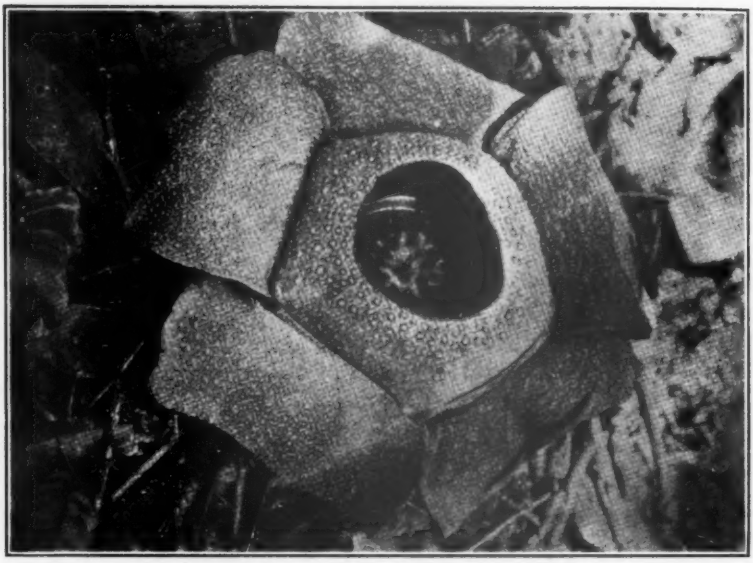
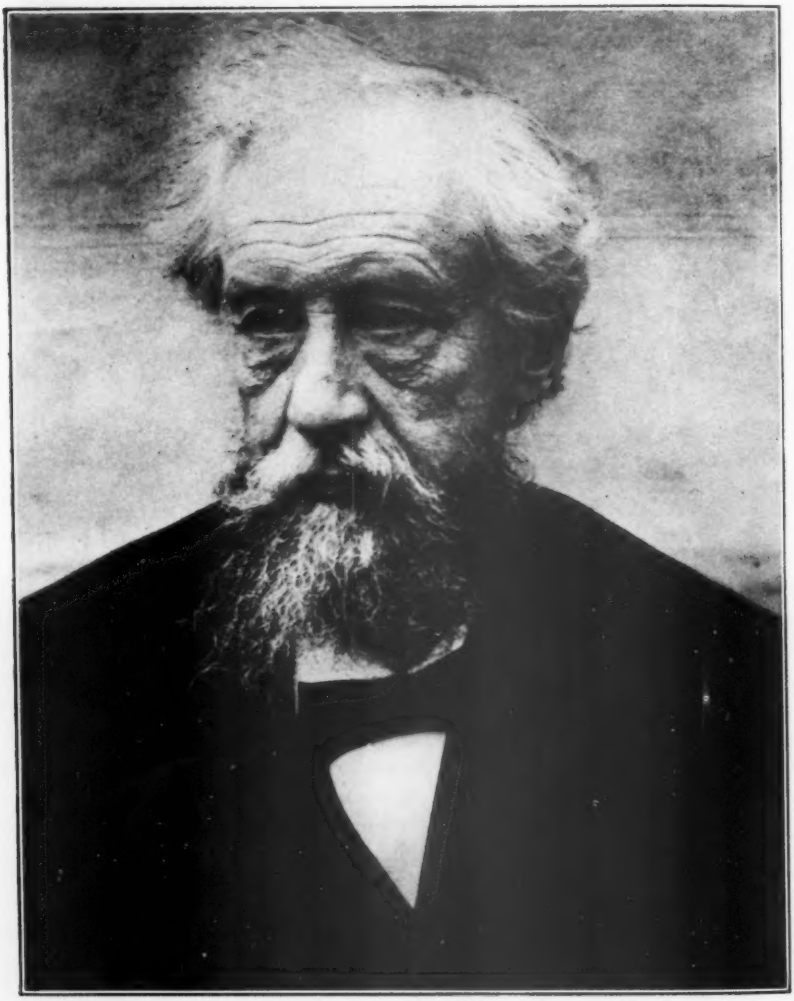


FIG. 7. Flower of *Rafflesia patma*.

The extraordinary variety of interests present in and about Java proved to be of great profit and joy to the Congress delegates. Java's interesting and modern tropical agriculture, its fauna and flora, its numerous active volcanoes and other geological situations, and its present races of men and the splendid evidences of prehistoric man, produced in the delegates a condition of general enthusiasm in which each scientific group was inclined to feel that it, somehow, held a distinct advantage over the others.

In closing this account it is a pleasure to the writer to mention again the splendid hospitality offered at all times to the delegates by the officials of the Netherlands East Indies government, and the Netherlands Science Council. The scientific value of such a Congress is admitted, but the words of His Excellency the Governor General were well taken when he said: "I attach still more value to the results which this Congress will bear in promoting closer understanding and good will between nations and individuals."

DEPARTMENT OF HORTICULTURE,
OREGON STATE COLLEGE.



HUGO DE VRIES
1848–

UNIV. OF WIOM.

BRIEF PAPERS

HUGO DE VRIES

(WITH ONE PLATE AND FOUR FIGURES)

One of the most distinguished of all botanists is HUGO DE VRIES. He studied in the University of Leiden in 1866 and afterwards in various German universities, first at Heidelberg in 1870 and then at Würzburg in 1871. At Würzburg, which was an international research center, he studied under the great plant physiologist, JULIUS VON SACHS, from whose laboratories have gone out so many scientists who have become famous. Besides DE VRIES, among these may be mentioned such other great men as PFEFFER, BREFELD, NOLL, STAHL, ELFVING, KLEBS, GOEBEL, G. KRAUS, F. DARWIN, and many others. SACHS exerted a great influence on the scientific thought and work of DE VRIES and many other investigators.

Professor DE VRIES occupied the chair of botany at the University of Amsterdam for many years. He was born at Haarlem, February 16th, 1848. His first paper, entitled "De invloed der temperature ob de levensverschijnselen der planten," appeared in 1870. Since the appearance of this paper other very important contributions have followed one another through the years in rapid succession. Many of the papers of DE VRIES have been collected by some of his students and published in seven large volumes under the title of "Opera e periodicis collata" (1918 to 1927). These volumes contain 189 of his contributions, of which the above mentioned paper is the first. The seven volumes included almost 4300 pages, which is only a part of the large amount of scientific work he has done. Professor DE VRIES has published his studies in four different languages. Some of his papers collected in "Opera e periodicis collata" are in the Dutch language, part of them in German, some in French, and the rest in English. Some of his contributions, however, are very extensive and therefore have been published in book form. Among these may be mentioned "Intracellulare Pangenesis," of which an English translation has appeared; and his great Mutationstheorie, 1901–1903, which appeared in German, in two large volumes. This work was soon translated into English. "Species and Varieties, Their Origin by Mutation" appeared in 1905. These and other important contributions appeared in book form and his "Species and Varieties" in more than one edition in English. A great service was rendered by DE VRIES since, in 1900, along with CORRENS and TSCHERMAK, he re-discovered MENDEL'S long forgotten work. The plate and fig. 1 are excellent likenesses of him from recent photographs.

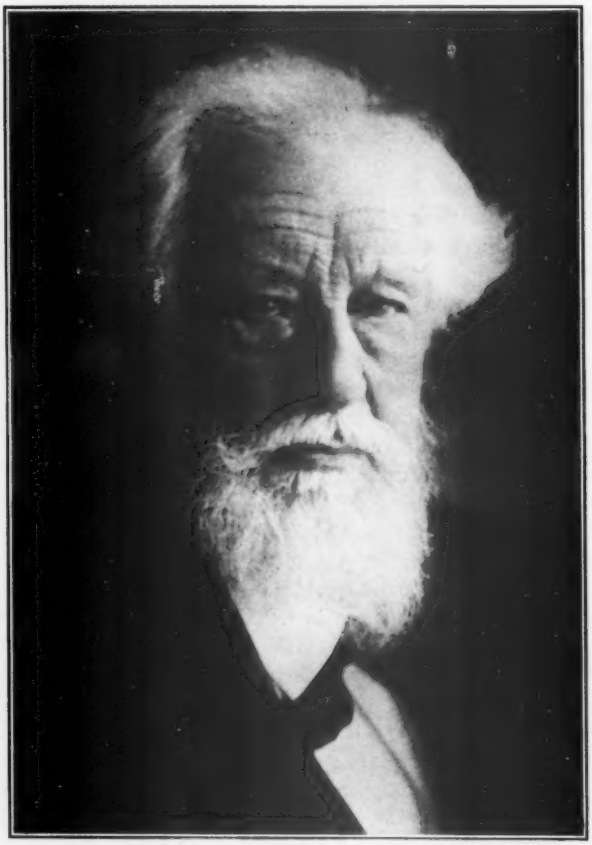


FIG. 1. Professor HUGO DE VRIES (recent photograph).

The first studies of Professor DE VRIES were concerned with plant physiology, in which subject he published many valuable papers. These contributions are of such unusual importance and are so well and favorably known that a mere mention of the titles of a few of them is sufficient. In addition to those contributions mentioned or referred to elsewhere by the writer in this paper, the results of his studies on plasmolysis, turgor, growth, osmosis, and other topics are of such far reaching importance as to place him among the most distinguished scientists of his day.

The importance of the distinguished work of DE VRIES is attested by the recognition accorded him by universities and scientific societies all over

the world. Some of the universities that have bestowed upon him the honorary degrees of Doctor of Science and of Laws are: Columbia University, and the Universities of Chicago, Pennsylvania, Cambridge (England), and Aberdeen (Scotland)

Many Academies of Science have honored him by membership as follows: The Dutch Academy of Science since 1878; Associate Member of the Royal Academy of Belgium, 1905; a Corresponding Member of the Institute de France, 1913; Ehrenmitglied der deutschen botanischen Gesellschaft, 1891; Ehrenmitglied der k. Akademie der Wissenschaften in Wien,

FIG. 2. Professor DE VRIES in his experimental garden.

1919; Corresponding member of the Prussian Academy of Sciences of Berlin, 1913; Foreign Member of the Royal Society, London, 1905; Member of the Academia dei Lincei Rome, 1902; and Corresponding Member of the Russian Academy of Sciences, 1925.

Various societies in the United States have honored him by membership for his scientific achievements. He is a Member of the American Philo-

sophical Society, Philadelphia, 1907; a Corresponding Member of the Academy of Natural Sciences, Philadelphia, 1903; Foreign Associate of the National Academy of Sciences of the United States of America, Washington, 1904; Corresponding Member of the Botanical Society of America, 1922; Honorary Member of the American Breeders Association, Washington, 1910; Honorary Associate of the Station for Experimental Evolution at Cold Spring Harbor of the Carnegie Institution of Washington, 1904; Professor DE VRIES was present at the opening of this station and delivered on that occasion an address on the "Aims of Experimental Biology."

Various medals have also been awarded to Professor DE VRIES as follows: The Darwin Medal by the Royal Society, London, 1906; The Veitsch Medal, by the Royal Horticultural Society, London, 1910; and the Linnaean Gold Medal by the Linnaean Society, London, 1929.

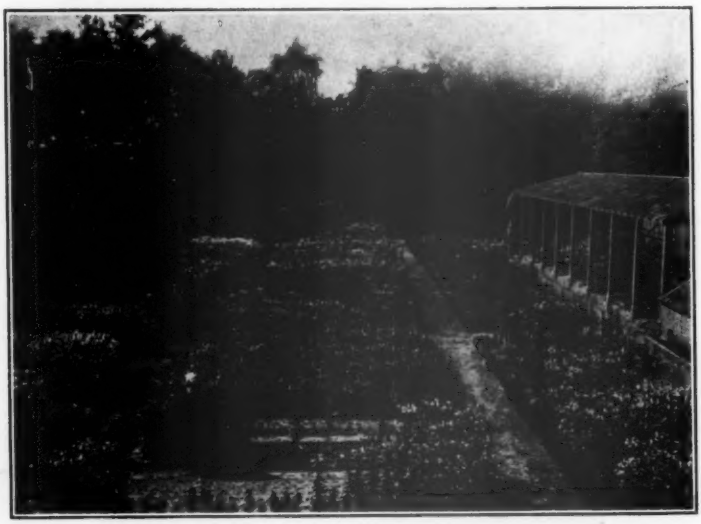


FIG. 3. Garden with *Oenothera* in bloom.

On the 16th of February, 1918, the Berichte der deutschen botanischen Gesellschaft sent him its greetings. He was then 70 years old and I quote two sentences only from that message, which are self-explanatory.

"Mit zwei Arbeitsgebieten der Botanik wird ihr Name für immer verknüpft bleiben. Waren es zunächst Fragen der physikalischen und chemischen Physiologie, wie des Turgordruckes, der Plasmolyse, der Mechanik des Zellwachstums, denen Ihre Hauptarbeit gegolten hat, so ist es später vor allem das Gebiet der Vererbungs- und Artbildungslehre auf dem

Sie bahnbrechend tätig waren und noch tätig sind." On his eightieth birthday in 1928 scientists from all over the world sent him messages and greetings.

Professor DE VRIES has visited the United States on three occasions. On two of these visits he delivered lectures at the University of California and the University of Chicago. His third visit to this country was in 1912 when he was invited to speak at the formal opening of the Rice Institute. While there he gave four lectures.

FIG. 4. Professor DE VRIES examining plants in his garden at Lunteren.

Figure 2 shows Professor DE VRIES in his experimental garden among the plants he is studying. In the photograph one can see a part of two of the flower beds in which the plants are grown, and at the right are to be seen four tall primrose plants whose tops are covered with paper sacks which are used in the pollination experiments.

Professor DE VRIES has been emeritus professor at the University of Amsterdam since 1918. He now resides at Lunteren, Holland. Here he has his garden, which, as shown in fig. 3, is full of *Oenothera* plants in bloom. The garden belongs to Professor DE VRIES and is part of the garden around his home. On the right side of fig. 3 is shown a portion of the garden enclosed with glass, and fig. 4 shows another view of the garden at Lunteren. It is in this part that most of the fertilizations and crossings of the experimental plants are made. Much of the material used by the recent students of Professor DE VRIES has been obtained from this garden for their studies.[1]

[1] The author is indebted to Professor DE VRIES for information concerning various points in this paper.

Two of the latest papers of Professor DE VRIES indicate his present scientific interests. These papers are: "Mutant races derived from *Oenothera lamarckiana semigigas*" and "Die latente Mutabilität von *Oenothera biennis* L."

Although past 81 years of age, Professor DE VRIES is still actively interterested and is diligently at work in his chosen field and continually adding to the long list of valuable contributions already made. His long and active life is an inspiration to two generations of younger investigators. Two great services he has rendered to science are first, that he has shown that mutations occur, and secondly, that in his investigations he has arrived at his results and conclusions by most careful experimentation.—F. M. ANDREWS, *Indiana University, Bloomington, Indiana.*

NOTE ON THE RELATION OF RATE OF RESPIRATION TO CHEMICAL COMPOSITION IN FRESH VEGETABLES

In a recent publication from this station,[1] the rates of respiration of some common vegetables during the first thirty hours after harvesting, were compared. Samples of the same material were prepared for analysis by careful drying, and with the exception of the phosphorus fractions[2] standard analytical procedures were used. It was thought that, although these vegetables represented different organs, and might naturally be expected to respire at different rates, there might be certain relationships between the rate of respiration and the chemical composition which would transcend the lines of demarcation laid down by function. In an attempt to detect such relationships, suggested, or even vaguely possible, the analytical data were plotted in a variety of ways together with the respiration figures. But we were unable to discover any relationships which ran consistently through the series.

Soluble nitrogen seemed to be directly related to respiration in the case of asparagus, green beans, okra, green onions, and carrots; with the other vegetables, the curve for this relationship was very irregular. Ether extract and lipoid phosphorus showed a nearly constant value for all the vegetables, without relation to respiration. The reducing-sugar values, which might have been expected to run parallel with the respiration curve, were extremely variable, as were also the starch values which might have been expected to bear an inverse relationship to respiration.

We realize that figures obtained from only ten samples do not constitute conclusive evidence one way or the other, and that there may be relationships here which our work fails to bring out.

The data are presented in the accompanying table. The weight of carbon dioxide evolved in 24 hours (from the second to the twenty-sixth after harvesting) per 100 grams dry weight, is taken in each case as the measure of the rate of respiration. The other figures are also on a dry weight basis.—MARJORIE P. BENOY and JAMES E. WEBSTER, *Oklahoma Agricultural Experiment Station.*

[1] BENOY, MARJORIE P. The respiration factor in the deterioration of fresh vegetables at room temperature. Jour. Agr. Res. 39: 75–80. 1929.

[2] WEBSTER, JAMES E. Phosphorus distribution in grains. Jour. Agr. Res. 37: 123–125. 1928.

TABLE I

ANALYSES OF VEGETABLES, AND CO_2 PRODUCTION PER 100 GRAMS (DRY WEIGHT) DURING 24 HOURS
PERCENTAGES ON DRY WEIGHT BASIS

DETERMINATION	ASPARAGUS	LETTUCE	GREEN BEANS	OKRA	GREEN ONION	CARROT	TOMATO	BEET	GREEN SWEET PEPPER	PIMENTO
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Dry matter in original material	8.34	6.16	7.67	13.66	9.34	13.91	6.13	9.51	8.01	9.38
Ash (dry basis)	7.84	17.58	7.66	6.54	9.73	13.17	10.38	19.75	7.05	9.51
Total phosphorus	0.70	0.49	0.53	0.38	0.34	0.45	0.50	0.28	0.43	0.55
Inorganic phosphorus	0.20	0.15	0.12	0.10	0.19	0.16	0.21	0.12	0.19	0.23
Lipoid phosphorus	0.05	0.05	0.04	0.04	0.07	0.05	0.04	0.05	0.05	0.05
Organic phosphorus	0.50	0.34	0.41	0.27	0.15	0.29	0.29	0.16	0.25	0.32
Total nitrogen	4.75	2.94	3.32	2.65	2.57	2.06	3.89	2.97	2.87	3.05
Insoluble nitrogen	0.94	.....	2.50	1.76	1.83	1.77	1.07	1.61	1.37	1.05
Soluble nitrogen	3.80	.....	0.82	0.89	0.74	0.28	2.79	1.36	1.50	1.99
Total sugars	35.88	8.92	24.65	12.30	22.81	12.01	39.58	8.63	32.07	36.03
Reducing sugars	33.21	5.68	24.65	11.57	20.99	8.20	39.58	5.58	31.95	31.66
Starch	1.54	1.61	4.97	18.75	0.77	6.03	1.12	0.86	5.02	1.15
Total carbohydrates	37.42	10.53	29.62	31.05	23.59	18.04	40.70	9.49	37.09	37.18
Ether extract	4.11	3.55	3.00	2.04	4.12	3.10	4.29	3.07	2.97	5.47
Weight CO_2 evolved in 24 hours per 100 grams dry weight	20.066	9.424	9.273	7.669	6.627	4.659	3.92	2.958	2.88	1.893

THE PHYSIOLOGICAL LABORATORY OF THE BOTANICAL INSTITUTE IN THE UNIVERSITY OF ZAGREB

A new Institute for Plant Physiology (fig. 1) has been established at the University of Zagreb, Jugoslavia, under the direction of Dr. VALE VOUK. The new laboratory is associated with the work in forestry, agriculture, pharmacology, and other scientific departments of the University, and also with the International Corn Borer Investigations Laboratory at Chicago.

At the dedication of the new laboratory on December 18, 1928, Dr. VOUK in his address reviewed the developments of plant physiology at the University of Zagreb and also the general history of the science in other countries.

The Institute comprises experimental gardens, greenhouse, laboratory, and library space. It was erected at a cost of approximately $20,000.—R. B. HARVEY, *University of Minnesota.*

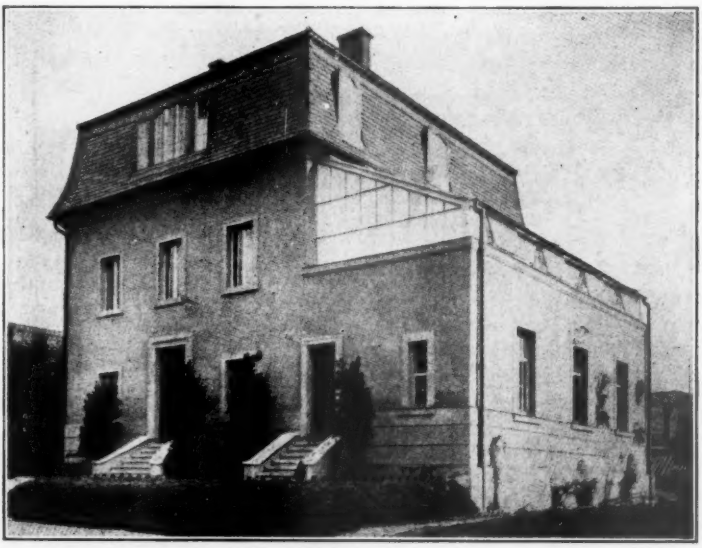


FIG. 1. The new laboratory for plant physiology at the University of Zagreb, Jugoslavia.

NOTES

The Annual Meeting.—The American Society of Plant Physiologists met at Des Moines, Iowa, on December 30 and 31, 1929, and January 1, 1930, for its sixth annual meeting. Dr. S. V. EATON, of the University of Chicago, presided over the meetings, assisted by Dr. A. E. MURNEEK, of the University of Missouri, vice president of the Society. The programs were well arranged and well organized, and nearly all of the papers were presented by the authors in person. Only a few papers were read by title only. The joint meeting with the Horticulturists on Tuesday morning at Des Moines, and with the Ecologists at Ames, were very pleasant occasions.

The secretary-treasurer, Dr. H. R. KRAYBILL, of Purdue University, presented his report for the fiscal year ending June 30, 1929, and the statement of the current condition of the treasury. The membership report showed a much larger increase than in either of the last two preceding years, but the increase in institutional subscriptions was not so great as during last year. However, the membership is approaching four hundred, and there are about 250 institutional subscriptions at the close of 1929. The treasury showed a very comfortable margin for 1929, and income for 1930 sufficient to maintain or even improve the publication service. It may be possible to bring out a new edition of the International Address List, which was first issued in 1925.

The banquet on Monday night, December 30, at the Savery Hotel, was attended by about 85 members and friends of the Society. The annual dinner has been featured for several years by the announcement of the awards of honors, which makes it one of the most interesting sessions of the meeting. The meeting of the Society for December, 1930, will be held at Cleveland, Ohio. By looking ahead and planning for larger things, we can make each annual meeting set a new and higher standard of achievement.

Life Membership Awards.—For the fourth time the Society has awarded life memberships to some of its most distinguished members as a memorial to Dr. CHARLES REID BARNES. The committee of award, with Dr. WALTER THOMAS, of Pennsylvania State College, as chairman, made two awards. The first one was to Professor GEORGE JAMES PEIRCE, for many years Plant Physiologist at Stanford University. Professor PEIRCE was born at Manila, Philippine Islands, in 1868. His scientific training was taken at Harvard, and at the Universities of Bonn, Leipzig, and Munich. His doctor's degree is from Leipzig in 1894. After two years of service at Indiana University, he went to Stanford in 1897, where he has given more than 30 years of his life to the advancement of plant physiology. His Text

Book of Plant Physiology was published in 1903, and a more recent book, The Physiology of Plants, in 1925. He has been interested in respiration, irritability, parasitism, and brine-inhabiting organisms. He has held various positions as special investigator of the effects of gas, smoke, and dust on vegetation, and was a member of the Advisory Committee to the Fuel Administration of California during the late war.

There was a bond of mutual friendship between Dr. BARNES and Dr. PEIRCE in the early days of plant physiology in the United States, and it seems very fitting that the award should be made to one of these early friends of BARNES.

The second award was made to Professor CHARLES A. SHULL, of the University of Chicago, who was at one time student assistant in Dr. BARNES's office, and who received his first instruction in plant physiology under Dr. BARNES in 1903–1906.

This action of the committee gives the Society five honorary life members, who constitute a living memorial to him who was the main inspiration of plant physiologists in the United States during the first decade of this century.

Stephen Hales Award.—The first award of the STEPHEN HALES Prize was also announced at the banquet. The Committee, consisting of Dr. J. B. OVERTON, chairman, Dr. C. R. BALL, and Dr. A. L. BAKKE, after consideration of many of the questions involved in the selection of candidates for this honor, finally made the award of the certificate and prize of $100 to Professor DENNIS ROBERT HOAGLAND, of California, for his excellent contributions and leadership in the field of plant nutrition. Professor HOAGLAND has been at the University of California for 17 years, and under his leadership the plant nutrition laboratory at California has made many important contributions, particularly with reference to the mineral nutrition of plants.

The certificate of award bears a handsome likeness of STEPHEN HALES, the first great experimentalist in the field of plant physiology. The plan of administration of the award contemplates an address by the recipient of the award before the Society at the following annual meeting.

The executive committee, on information from the secretary-treasurer as to the funds available for a STEPHEN HALES award on November 1, 1929, voted a second award to be made at the annual meeting at Cleveland in 1930.

The Charles Reid Barnes Life Membership Fund.—The Society, at the recommendation of the executive committee, has taken favorable action on a plan to create a permanent CHARLES REID BARNES Life Membership Fund

to replace ultimately the present temporary financing. The plan adopted is to establish such a permanent fund to which individual gifts may be made. In addition, any of the life membership funds vacated by the death of life members are to be put into the permanent fund until the total of such private gifts and vacated funds reaches the sum of $2000. This fund will then be known as the CHARLES REID BARNES Life Membership Fund, the income of which will be employed in the same manner as is the income from the present temporary fund.

Individuals, some of whom have expressed a desire to share in the creation of this permanent fund, should now avail themselves of the opportunity. Checks may be sent at any time to Dr. H. R. KRAYBILL, Purdue University. Or, the purchase of a life membership by those who can afford to make the investment, will now give the investor his official journal for life, and finally assist in the creation of this permanent fund in honor of Dr. BARNES. There is no better way to aid than by the purchase of life memberships. They can be paid in installments, but do not become operative until paid in full.

The Stephen Hales Prize Fund.—This fund is slightly less than $1000. It ought to be larger, as it requires not less than $1000 to produce the amount of the prize every two years. The funds are now invested in bonds yielding above the average interest. When reinvested it is doubtful if conditions will warrant renewal at the present rate. There are now about four times as many members in the Society as the number who contributed the $991.75 which constitutes the fund. Many small gifts are more desirable than a few large ones. It is not improbable that members will be offered the privilege of sharing in the enlargement of this fund. A 50 per cent. increase would insure the award as at present planned, and would permit increases in the amount of the biennial award, or would make more frequent awards possible.

General Endowment Fund.—The Society voted at the Des Moines meeting that a general endowment fund would be a desirable thing. At present it would hardly be possible to undertake publication of papers which demand colored plates for proper presentation of results. It usually costs several hundred dollars for a single colored plate. Moreover, as PLANT PHYSIOLOGY serves a larger and larger membership, there is the possibility that the size of the volume would finally be limited by the income, so that worthy contributions could not be given prompt publication. Endowment funds protect an organization from such difficulties, and make possible types of service beyond that of the ordinary journal. The finance committee is authorized to plan for the beginning of an endowment fund. If any mem-

ber believes that PLANT PHYSIOLOGY should, when necessary, use colored plates, or undertake other extraordinary services in publication, a gift to the general endowment fund, even though small, will help toward the achievement of such services. It is hoped that every member will take an interest in these funds, and will try to share, as fortune permits, in the privilege of founding these financial bulwarks of the Society. Any gift sent to the secretary-treasurer, or to the members of the finance committee, should be plainly designated to some particular fund. Every gift will further the purposes of the Society and help it to give a better service to its members.

Travel to the International Botanical Congress.—Some of the members of our Society will be going to Cambridge next summer. This notice is to call attention to the service of the Student Third Cabin Association. It offers a means of going and coming that may interest many of us. It is a special Tourist Third Cabin on the Holland-America Line ships which was created by two Yale students six years ago. It differs from other Tourist Third Cabins in being maintained entirely for college people and those with whom they naturally associate. In addition to most congenial fellow passengers on the ocean, it offers cabins, decks and public rooms that were formerly second class on the steamers Rotterdam, New Amsterdam, Volendam, and Veendam, and remarkable Tourist Third Cabin accommodations on the new Holland-America Line flagship Statendam. The service on these vessels is all that could be desired, the food is first class, well prepared and simply served, and everything is kept scrupulously clean.

Sailings are each Saturday from New York. The ships of this line call at Plymouth in England going to Europe, and leave from Southampton on the return voyage. In France, the port both ways is Boulogne-sur-Mer, two and one half hours from Paris. The other European port is Rotterdam, Holland, the gateway to Central Europe.

The parties crossing are provided with a host and hostess, a lecturer and college orchestra, and a loan library on all of the larger STCA sailings. A guide book is obtainable, called the Hand-Me-Down, that is one of the best for European travel, and there is a complete travel department that can help individuals or groups to plan anything desired in the way of travel experience while abroad. Information on renting Drive-yourself cars in Europe will be furnished. There is no charge for such service except for the Hand-Me-Down.

A number of single, double, and four berth cabins have been reserved on each sailing in June, July and early August with corresponding returns for people going to the Congress. Those who are planning to go should investigate this plan, as it is less expensive than many, and leaves more

money to spend abroad. Full information will be furnished to any one, on sending inquiry to the Student Third Cabin Association, Holland-America Line, 24 State St., New York City.

Dr. Felix Kotowski.—We regret to announce that one of our foreign members has been claimed by death. The following statement concerning the life of Dr. FELIX KOTOWSKI has been prepared from data furnished by Dr. J. GOLINSKA, a colleague, and by W. W. BRIERLEY, Secretary of the General Education Board, New York.

Dr. KOTOWSKI was born on May 18, 1895, in Grabova, near Radom in central Poland. He was educated in Poland, and was a student in the Division of Biological Science and Agriculture in the University of Krakow from 1913 to 1918. He received the degree of Doctor of Philosophy from the Jagellon University in Krakow in 1919. During his student days he was Assistant in the Institute of Soil Management and Plant Cultivation in the University, 1917–1918. After graduation, and until 1922, he was assistant in the Horticultural Department of the State Scientific Institute at Pulawy. In January, 1922, he was appointed Professor of Olericulture at the College of Agriculture, Warsaw, and Director of the Institute of Olericulture and Vegetable Breeding at Skierniewice, near Warsaw. This position he held until July, 1926, at which time he was granted a fellowship for study in the United States by the International Education Board. During the period of the fellowship, to September, 1927, he worked at the University of California, Davis, California, in the Division of Truck Crops, under the supervision of Professors H. A. JONES and J. T. ROSA. The results of the year's work have been published in the Proceedings of the American Society for Horticultural Science, 1926, and in PLANT PHYSIOLOGY, 1927. While in the United States he also visited the experiment stations at Ithaca and Geneva, New York, to familiarize himself with their investigations of vegetable crops. He returned to Poland by way of the Pacific, in order to visit experiment stations in Hawaii, Japan, Ceylon, and India.

Dr. KOTOWSKI was very industrious, and labored to secure a universal scientific education. During the ten years of his scientific career, he published about 40 papers on the morphology, physiology, and breeding of vegetable plants. He was the author of a text-book of horticulture, and several popular publications on different horticultural questions. He was also one of the leaders in organizing the olericultural experimental work in Poland.

He died on July 9, 1929, of blood-poisoning caused by a scratch of the lower lip. The untimely death of Professor KOTOWSKI at the age of 34

years is a great loss to Polish horticultural science. His students have lost a brilliant guide, and a real friend.

Back Numbers Wanted.—Volume 2 of PLANT PHYSIOLOGY is practically exhausted. The limiting factor is no. 4, the October issue, 1927. Members and subscribers are urged to examine their files, and if duplicates of this number are found, the Secretary-treasurer would like to purchase copies of this number to complete broken volumes. Any one willing to dispose of this particular number should write to Dr. H. R. KRAYBILL, Purdue University, Lafayette, Indiana. Cooperation of the members and subscribers in this matter will be greatly appreciated.

Pathology of Protoplasm.—The third volume of Protoplasma-Monographien is entitled "Pathologie der Pflanzenzelle." Part I bears the title "Pathologie des Protoplasmas." The author is Dr. ERNST KÜSTER, of Giessen. The discussion is presented in two chapters, one on Formwechsel, the other on Strukturwechsel. The character of the material presented is such that it should hardly be called pathology. It really presents some interesting phases of the physiology of protoplasm, and plant physiologists will find the monograph worth reading for a better understanding of protoplasmic responses to all kinds of environmental stimulations. Over 500 references to the literature are included at the close. The publishers are the Gebrüder Borntraeger, W 35 Schöneberger Ufer 12 a, Berlin, Germany. The price of the volume bound in cloth is 15 M.

Problem of Krakatao.—The problem of the revegetation of Krakatao after the destructive eruption of August 27, 1883, has been given a refreshingly critical treatment by Dr. C. A. BACKER, formerly government botanist for the flora of Java. BACKER takes sharp issue with the dictum of TREUB that the explosion completely destroyed the original vegetation of the island. He shows how inadequate all of the investigations have been, and how the opportunity to study this interesting problem of revegetation was forever let slip.

The book has 12 chapters and 299 pages. It is published by the author, but can be purchased from Martinus Nijhoff, The Hague, Holland. The price, bound, is 9 guldens, plus postage.

Soil Acidity.—An excellent monograph on soil acidity by Dr. H. KAPPEN has appeared from the press of Julius Springer, Berlin. "Die Bodenacidität" contains fifteen chapters, as follows: Nature of acidity of mineral soils; soil reaction; determination of soil reaction; behavior of acid

soils toward acids and bases—their neutralization or buffer capacity; behavior of acid soils toward solutions of salts (hydrolytic, exchange, and active acidity, etc.); absorption power of acid soils; meaning of acidification for physical soil characters; influence of reaction on the microorganisms of the soil; the plant physiological meaning of soil reaction; occurrence and distribution of soil acidity; influence of manures on soil acidity; the prevention of acidity injuries by liming; and the use of artificial fertilizers on acid soils.

There are 363 pages, 35 text figures, and one colored plate. This work will be quite valuable to the student of soil acidity problems. The price unbound, is 36 M., bound, 38.80 M.

International Critical Tables.—Volume VI of this great work was issued late in 1929 by the McGraw-Hill Book Co. The general nature of the contents of this volume was indicated in PLANT PHYSIOLOGY **4**: 295. 1929. The following data are included: X-ray, electronics and gas conduction, dielectric properties, electrical conductivity and resistivity, pyro- and piezo-electricity, thermoelectricity, transference numbers of electrolytes in aqueous solutions, electrolytic electromotive force, electrical and optical properties of SiO_2, magnetism, atmospheric electricity, terrestrial magnetism, and acoustics. The work is immensely valuable to all fields of science. The final volume is due sometime during 1930.

Plant Ecology.—The new text-book on Ecology by Dr. J. E. WEAVER, of Nebraska, and Dr. F. E. CLEMENTS, of the Carnegie Institution, is a notable attempt to provide a good systematic survey of this field. Plant physiologists should find this volume particularly welcome as a text in Ecology, as it is the best attempt so far to give ecology a balanced physiological setting. We should all be interested in the field aspects, as well as the laboratory aspects of our field, for plant behavior does not cease to be physiological just because it occurs in the open rather than under glass or in a laboratory; and all plant physiologists will be better physiologists by knowing something of the vegetational phenomena of the earth. The parts particularly valuable to the physiologist are those chapters and sections that deal with the soil, underground plant parts, adaptation, germination of seeds, dormancy, summation of temperatures, tolerance, effects of duration of light, etc.

This book is so far superior to anything else available for the study of ecology as to leave it in a field by itself. The authors and publishers have prepared a book that will no doubt have a deep influence upon the study of plant life. The work is sold at $5.00, and familiarity with its contents will prove a good investment of time and money. Orders for it can be sent to the McGraw-Hill Book Co., New York.

Plant Competition.—Publication no. 398 of the Carnegie Institution, Washington, D. C., entitled "Plant Competition, an Analysis of Community Functions," is by Dr. F. E. CLEMENTS, Dr. J. E. WEAVER, and Dr. HERBERT C. HANSON. It is an experimental analysis of the competition of plants in the plant community, and the plant reactions which come from such competition. Some new methods have been devised for a comprehensive study of the phenomena, and studies are made of natural communities, crops, and greenhouse controls. Many of the results are of great value to the physiologist.

The first section develops the history of the competition concept, following which are sections dealing with transplant cultures in subclimax prairies and true prairies, with supplementary studies of prairie competition. Competition in prairie-woodland tension zones and in cultivated fields are considered, and the relative importance of the various factors in competition. The last two sections deal with functional studies in control cultures, and the nature and rôle of competition.

There are 32 plates, a few colored to distinguish root systems in competition. Including a lengthy bibliography, the book contains 340 pages. It is an important contribution to the general problem of competition among organisms. It can be purchased from the Carnegie Institution for $3.25, or $4.25 in cloth binding.